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On the demography of *Echinochloa crus-galli* and the effect of post-dispersal seed predation in maize fields

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Summary

Integrated weed management, aims to maintain the weed populations at a manageable level by using a diverse array of tools, i.e. biological, chemical, physical and cultural measures. The goal of this study was to identify the demography of the weed species *Echinochloa crus-galli* (L.) P. Beauv and the effect of post-dispersal seed predation in maize fields to test the weed control potential of seed predators as a conservational biological control measure. Not only do seed predators consume seed on the soil surface, before seeds burial into the soil; they also reduce the input of newly produced seeds into the seedbank, thus increasing seed mortality. To reach our study goal, three main obstacles were addressed: seed predators behaviour, weed species *E. crus-galli* demography and seed predators effect on the demography of the weed. Therefore, we tested seven main objectives. First, to identify the behaviour of seed predators, we estimated the level and response of seed predation to different weed seed densities of *E. crus-galli* in autumn. Second, we tested whether seed predation in autumn is a good estimate of seed predation from seed shed until the following spring (autumn plus winter). The third and fourth objectives aimed to understand *E. crus-galli* demography without seed predation. Specifically, the third objective tested whether relationship between the number of seeds per panicle dry weight or per panicle length can be used to simplify the measurement of the seed production of *E. crus-galli*. Furthermore, we included the effect of factors that could influence this relationship, such as the time of seedling emergence, the density of *E. crus-galli*, the control intensity of other weeds, seed predation and field. The fourth objective tested the role of density-dependent regulation in *E. crus-galli* demography. The last three objectives addressed the effect of seed predation on the demography of density populations of *E. crus-galli*: the fifth tested whether seed mortality by seed predation will lower the density of seedlings; the sixth, whether density-dependent seedling mortality and fecundity will compensate for the lower number of seedlings; and the seventh, whether seed predation will not affect the final constant level of seed production per unit area, but the level will vary between fields.

All Objectives were tested in an agroecosystem in north-eastern Germany. Here, two experimental approaches – one short-term and one long-term - were conducted on three minimally tilled maize fields that had a history of three consecutive years of maize. In the short-term experiment, which tested the first two objectives, different densities of seeds of *E. crus-galli* were applied on seed trays, such that seeds were exposed to seed predators during autumn in 2014 and 2015 (August until corn harvest in September - October). In the long-term

experiment, which tested another part of objective two and remaining objectives, different densities of *E. crus-galli* seeds were applied to plots in autumn 2014. Halve of the plots were enclosed by a plastic frame to prevent the access of seed predators. In the following season in 2015, the number of seedlings, adult plants, and seed production m^{-2} were determined in the plots. To test the third objective, a few days before maize harvest, all panicles were removed from the fields and dried panicles were weighed and panicle length was measured, and for a subsample of panicles, the number of seeds was counted manually.

For the first two objectives addressing seed predators behaviour, results showed that in autumn 2014, the level of seed predation and the response to seed density differed between fields. In autumn 2015, in the three fields, a high number of seeds were removed via seed predation, when rodents dominated the seed predator assemblage. The response to seed density was density independent, as seed predation during the winter partially resulted in an increased level and a density-independent response to seed density in all fields. Thus, seed predation in autumn does not reflect seed predation from seed shed until the following spring. Analysis to test the third objective to simplify the measurement of seed production in *E. crus-galli*, showed that panicle dry weight ($R^2 = 0.92$) predicted the number of seeds per panicle better than panicle length ($R^2 = 0.69$). The other tested factors, except for "field" and "seed predation" had no effect on these relationships. The relationships between seed number and panicle dry weight found in this study closely resembled the results found in an earlier study. Based on our findings, we emphasize that both plant traits were appropriate to use in estimating seed production, depending on the users´ demand for precision and available resources for evaluating sustainable weed management strategies. Results on the fourth objectives showed that the demography of *E. crus-galli* was regulated by density-dependent processes. Density-dependent mortality hampers the seedling emergence, seedling survival and per capita fecundity of *E crus-galli* and it results in a constant level of final seed production. Seed predators effect on the demography of *E. crus-galli* showed that while the number of seedlings was reduced, but density-dependent processes during seedling emergence, seedling survival and fecundity per capita compensated for the losses. Seed production per unit area was stable among all population densities of *E. crus-galli*. Results on the seventh objective showed that the final seed production was affected by in-field conditions. All initial population densities of *E. crus-galli,* either in the presence or absence of seed predators, increased from one vegetation period to the next.

In summary, the weed species *E. crus-galli* demography is regulated by density dependent processes and, thus, seed predation as a single control measure fails to limit the

growth of population densities. In combination with other control measures that target the seedling survival and fecundity, however, seed predation may contributes to weed control by lowering the input into the seedbank and, thus, the distribution of the seeds. Furthermore, *E. crus-galli* is able to compensate weed seedlings that escapes from failed weed management in maize crops. The long-term effect of integrated control measures on the seedbank of *E. crus-galli* has not been simulation, however, this study provides data on the fully parametrized life-cycle of *E. crus-galli* that will support future applications to simulate the long-term effect.

Zusammenfassung

Die Integrierte Unkrautkontrolle bedient sich biologischer, chemischer, physikalischer und kultureller Maßnahmen, um das Wachstum der Unkrautpopulationen zu einem kontrollierbaren Maß zu begrenzen. Ziel dieser Dissertation war es die Demographie des Unkrautes *Echinochloa crus-galli* (L.) P. Beauv. und den Einfluss von Samenprädation auf Populationsdichten von *E. crus-galli* in Maisfeldern abzubilden. Ergebnisse geben Aufschluss über das Potential der Samenprädatoren zur Unkrautkontrolle als eine biologische Maßnahme die natürlich auf den Feldern vorkommt. Samenprädatoren ernähren sich von Unkrautsamen auf der Bodenoberfläche bevor sie in den Boden einsinken. Sie reduzieren damit den Eintrag von neu produzierten Samen in die Bodensamenbank und erhöhen die Sterblichkeit der Samen. Um das Ziel der Arbeit zu erreichen, müssen drei Fragestellungen analysiert werden; Verhaltensweisen der Samenprädatoren, die Demographie von *E. crus-galli* und der Einfluss von Samenprädatoren auf die Demographie von *E. crus-galli*. Dafür werden sieben Zielsetzungen getestet. Um Aufschluss über die Verhaltensweisen der Samenprädatoren zu erlangen, wurden im Herbst das Niveau und die Reaktion der Samenprädation auf unterschiedliche Aussaatdichten der *E. crus-galli* getestet. In der zweiten Zielsetzung wurde getestet, ob mit der im Herbst gemessenen Samenprädation auch die Samenverluste vom Herbst bis zum nächsten Frühjahr (Herbst und Winter) abgeschätzt werden kann. Die dritte und vierte Zielsetzung dient dazu die Demographie von *E. crus-galli* unabhängig von Samenverlusten durch Samenprädatoren zu verstehen. Die dritte Zielsetzung erfolgt, um die Messung der Samenproduktion von *E. crus-galli* zu vereinfachen. Dazu wurde der Zusammenhang zwischen der Anzahl Samen pro Rispe und der Trockenmasse oder Länge der einzelnen Rispe getestet. Weiterhin wurde die Stabilität dieser Zusammenhänge mit folgenden Faktoren getestet: unterschiedliche Auflaufzeitpunkte, Aussaatdichten von *E. crus-galli*, genetischen Populationen, Intensität der Kontrolle anderer Unkräuter, Samenprädation und der Einfluss der Felder. Die Regulierung der Demographie von *E. crus-galli* durch dichteabhängige Prozesse wurde in der vierten Zielstellung und der Einfluss der Samenprädation auf die Demographie von *E. crus-galli* in den letzten drei Zielstellungen untersucht. Mit der fünften Zielstellung wurde getestet, ob Samenverluste durch Samenprädation zu einer Verringerung der Keimlinge führen und mit der sechsten, ob die Dichteabhängige Prozesse zu einer Kompensation der Samenverluste führen. In der siebten Zielstellung wurde getestet, ob die Höhe der Samenproduktion auf Populationsebene von den jeweiligen Feldern und nicht von der Samenprädation beeinflusst wird.

Die Zielsetzungen wurden in einem Agrarökosystem in Nordostdeutschland getestet. Dafür wurden jeweils zwei Experimente, ein Kurz- und Langzeitexperiment, auf drei Maisfeldern installiert. Diese Maisflächen wurden mit minimaler Bodenbearbeitung bewirtschaftet und befanden sich seit mindestens drei Jahren in Folge im Maisanbau. Das Kurzzeitexperiment wurde installiert, um die ersten beiden Zielsetzungen zu testen. Dafür wurden Samen von *E. crus-galli* in unterschiedliche Dichten auf mit der Bodenoberfläche abschließende und mit Erde befüllten Schalen gesät. Das Kurzzeitexperiment fand jeweils im Herbst 2014 und 2015 (August bis zur Maisernte im September bzw. Oktober) statt. Das Langzeitexperiment wurde installiert um einen Teil der Zielsetzungen zwei und alle weiteren Zielsetzungen zu testen. Dazu wurden im Herbst 2014 unterschiedliche Dichten von *E. crus-galli* in Parzellen ausgesät. Ein Teil dieser Parzellen wurde mit Rahmen aus Plastik von dem Einfluss der Samenprädatoren geschützt. In der folgenden Saison 2015, wurden die Anzahl Keimlinge, adulte Pflanzen und Anzahl der neu produzierten Samen m^{-2} in den jeweiligen Parzellen gemessen. Um die dritte Zielsetzung zu testen wurden alle Rispen von *E. crus-galli* vor der Maisernte aus dem Langzeitexperiment entfernt, ihre Trockenmasse gewogen und die Rispenlänge bestimmt. Von diesen Rispen wurde eine Teilprobe entnommen, um die Anzahl Samen der Rispen zu bestimmen.

Die Ergebnisse zu ersten beiden Zielsetzungen zeigen, dass sich im Herbst 2014 das Niveau und die Reaktion der Samenprädatoren auf unterschiedliche Aussaatdichten der *E. crus-galli* zwischen den Feldern unterschied. Im Herbst 2015 dagegen, war das Niveau der Samenverluste sehr hoch und die Reaktion auf Aussaatdichten dichteunabhängig. Im Jahr 2015 dominierten granivore Mäuse die Zusammensetzung der Samenprädatoren. Bei verlängerter Verfügbarkeit der Samen bis zum nächsten Frühjahr, stieg das Niveau der Samenverluste leicht an und die Reaktion der Samenprädatoren auf die unterschiedlichen Dichten wurde in allen Feldern dichteunabhängig. Samenprädation im Herbst ist also nicht mit der Samenprädation über dem Winter vergleichbar. Analysen zur Vereinfachung der Messung der Samenproduktion (dritte Zielsetzung) zeigen, dass durch die Trockenmasse der Rispen (R^2 = 0.92) die Anzahl Samen pro Rispen akkurater und präziser berechnet wird als durch die Rispenlänge (R^2 = 0.69). Andere Faktoren, ausgenommen der Felder und Samenprädation hatten keinen Einfluss auf die Zusammenhänge zwischen Anzahl Samen pro Rispe und Eigenschaften der Rispe. Ausgehend von diesen Ergebnissen, sind beide Eigenschaften der Rispen für die Berechnung der Anzahl Samen pro Rispe nützlich. Die Wahl zwischen den beiden getesteten Rispeneigenschaften zur Bestimmung der Anzahl Samen pro Rispe ist abhängig von der geforderten Genauigkeit und verfügbaren Ressourcen zur Aufnahme der

Daten. Ergebnisse zur vierten Zielsetzung zeigten, dass dichteabhängige Prozesse die Demographie von *E. crus-galli* regulierten. Dichteabhängige Prozesse verringerte die Keimung, Überleben der Keimlinge und die Samenproduktion pro Pflanze und mündeten in einer konstanten Höhe der Samenproduktion in allen Populationsdichten. Samenverluste durch Samenprädation beeinflussten die Demographie von *E. crus-galli*, sodass die Anzahl der Keimlinge reduziert wurden. Diese verringerte Anzahl wurde jedoch durch die dichteabhängigen Prozesse während der Keimung, Überleben der Keimlinge und Samenproduktion pro Pflanze kompensiert und mündete ebenfalls in eine konstante Samenproduktion auf Populationsebene. Ergebnisse zur siebten Zielsetzung zeigen, dass die Höhe der Samenproduktion nicht durch Samenprädation, jedoch durch die jeweiligen Bedingungen im Maisfeld beeinflusst wurden. Alle ursprünglich ausgesäten Populationsdichten von *E. crus-galli* konnten unabhängig von Samenverlusten durch Samenprädation zwischen zwei Vegetationsperioden wachsen.

Somit wird die Demographie von *E. crus-galli*, einer der bedeutendsten Unkrautart im Maisanbau durch dichteabhängige Prozesse reguliert. Samenverluste durch Samenprädation werden kompensiert und somit hat Samenprädation als alleinige Maßnahme kein Potential das Wachstum der Populationsdichten von *E. crus-galli* zu kontrollieren. In Kombination mit anderen Maßnahmen, die das Überleben der Keimlinge und die Samenproduktion pro Pflanze kontrollieren, kann Samenprädation jedoch zur Unkrautkontrolle beitragen. Samenprädation reduziert den Eintrag von neuen Samen in die Samenbank und die Ausbreitung der Samen. Die Kompensationsfähigkeit von *E. crus-galli* ist weiterhin bedeutend für Maßnahmen die Keimlinge unzureichend bekämpfen konnten. Überlebende Keimlinge kompensieren den Verlust durch dichteabhängige Prozesse. Der Langzeiteffekt von integrierten Maßnahmen auf das Wachstum der Samenbank von *E. crus-galli* wurde in dieser Arbeit nicht simuliert, jedoch liefert diese Dissertation mit der Parametrisierung des Lebenszyklus von *E. crus-galli* eine wichtige Grundlage für diese Simulationen

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Abbreviations

a.i.	Active ingredient
c.f.	confer
df	Degrees of freedom
DFG	German Research Foundation
e.g.	exempli gratia
et al.	et alii
f	Per capita fecundity
GLM	Generalized Linear Regression Model
i.e.	id est
IWM	Integrated Weed Management
K	Potassium
LM	Linear Regression Model
N	Nitrogen
n	Number of repetitions
PhD	Doctor of Philosophy
P	Phosphor
pred	Post-dispersal seed predation
r	Seedling emergence
s	Seedling survival
SE	Standard error
sp	Seed production per unit area

Chapter 1

General Introduction

General Introduction

Weed control in agroecosystems is dominated by the use of herbicides. Because herbicides are effective and economical, they are intensively used in farming practices. However, their use, along with the concomitant loss of weed species, negatively affect farmland biodiversity (Boatman *et al.*, 2004; Taylor *et al.*, 2006), and residues persist in soil, water and air (Hvězdová *et al.*, 2018). Moreover, as weed species evolved in their resistance to some modes of action, herbicides lose their effectiveness over time (Heap, 2019). To reduce the negative effects related to herbicides, political guidelines propose to minimise herbicide application using integrated weed management and to develop new tools for this approach (European Directive 2009/128/EC on the sustainable use of pesticides).

1.1 Integrated weed management

Integrated weed management (IWM) aims to reduce the reliance on herbicides by using a diverse array of tools – i.e. biological, chemical, physical and cultural – to maintain weed populations at a manageable level (Barzman *et al.*, 2015). The use of each tool on its own would fail to manage weeds, but in particular combination they can reduce weed population densities (Liebman & Gallandt, 1997). IWM was established in the 1950s as a reaction to the intensive use of herbicides. Although weed research is, to date, mainly herbicide related, environmental concerns and weed evolution and adaptation compel the weed researcher to have an increased interest in IWM (Harker & O'Donovan, 2013; Westwood *et al.*, 2018). This rise in scientific interest in subjects dealing with IWM can be identified by the almost exponential increase of articles within this framework (i.e. 1 article in 1989 vs 205 published articles in 2018) (Web of Science Citation Report, 2019b). Especially in countries such as the United States, Canada and Australia that are facing a rapid evolution of herbicide-resistant weed species and therefore a declining number of available herbicidal modes of action, research activities exploring IWM have increased (Harker & O'Donovan, 2013; Heap, 2014). If one considers the number of published articles on IWM, research in Europe is less prominent (Web of Science Citation Report, 2019b). Nonetheless, researchers in France and Italy, followed by Germany, are exhibiting some activities in this area (Harker & O'Donovan, 2013). Novel control measures – such as the use of biopesticides, automation technologies for site-specific weed control, crop cultivars improved for better weed competition or using biological control to reduce weeds – are promising tools in a sustainable weed management approach (Westwood *et al.*, 2018). The biological control of weeds is defined by the introduction of a natural enemy of the pest from another geographical area. In contrast, ‘conservational’ biological control maintains and

enhances the abundance of naturally occurring enemies of the pest, thereby regulating the populations of pests, including weed species. To advance the use of conservational biological control measures, knowledge of the behaviour of the natural occurring enemies, the life-cycle of the weed itself and the interaction between both is necessary (Mortensen *et al.*, 2000; Swanton *et al.*, 2008).

1.2 Post-dispersal seed predation

This study focused on post-dispersal seed predation in agroecosystems, representing a potential agent in conservational biological control. Ecological and agricultural science community interest in weed seed predation steadily increased over the past two decades, growing from 98 published articles in 1997 to 2007, to 226 published articles in 2007 to 2017 (Web of Science Citation Report, 2019a). The main scientific contributions in this field originated from research groups in the USA, Canada, Germany, France, Czech Republic, UK and Austria. Research on weed seed predation aims to understand the effect of seed predators on the weed seeds before they contribute to the weed seedbank and germinate in the next vegetative period. To identify the biology and ecology of post-dispersal seed predation, the scientific community mainly addresses three different aspects: (i) multi-trophic interaction between seed predators, weed seeds and second-order predators; (ii) in-field and landscape management; and (iii) effect of seed predation on weed demography (Petit *et al.*, 2018).

Naturally occurring post-dispersal seed predation can limit the input of newly shed seeds to the seedbank by 8–70 % (Davis *et al.*, 2011). The time of seed predation is restricted to the period when seeds are available on the soil surface, between the time when seeds are shed and when the available seed on the ground is buried into the soil (Westerman *et al.*, 2009). In temperate regions, main seed predators are carabid beetles (Honek *et al.*, 2003; Frei *et al.*, 2019) and granivorous rodents (Daedlow *et al.*, 2014; Fischer *et al.*, 2018; Tschumi *et al.*, 2018b), but not voles (Fischer *et al.*, 2018). From the farmer's perspective, rodents are rather associated to disservice (consumption of crop seeds) than to service (consumption of weed seeds). Indeed, service and disservice have been reported for rodents in spring cereal fields (Tschumi *et al.*, 2018a). In particular, disservice was linked with the presence of voles but not with granivorous species (Fischer *et al.*, 2018). However, given that rodents and even carabid beetles emigrate from semi-natural habitats when the crop has been established (Pfiffner & Luka, 2000; Aschwanden *et al.*, 2007; Tschumi *et al.*, 2018a), seed predators are hardly relevant for crop yield losses.

Seed predator biology determines the time period for seed consumption. For instance, carabid beetles are either active in spring or autumn (Kromp, 1999) and hibernate in temperatures below 8 °C (Saska *et al.*, 2010), whereas rodents are active the whole year. Weed preferences may change the weed assemblage in the field, as seed traits such as size (Honek *et al.*, 2007) and lipid content (Gaba *et al.*, 2019) drive the feeding behaviour of seed predators.

To date, research on in-field management mainly addressing tillage and crop type shows increasing predation rates in no-till fields (Cromar *et al.*, 1999; Baraibar *et al.*, 2009; Petit *et al.*, 2017) as well as in fields with crop vegetation cover (Heggenstaller *et al.*, 2006; Eyre *et al.*, 2013; Labruyere *et al.*, 2016) and cover crops (Gallandt *et al.*, 2005; Ward *et al.*, 2011). The complexity of the landscape – small fields surrounded by a matrix of non-crop habitats such as hedgerows and woodlots – affects seed predation (Menalled *et al.*, 2000; Martin *et al.*, 2020). Non-crop habitats serve as overwintering sites or refuges for seed predators. Thus, there is growing evidence that a clever combination of in-field and landscape management can accelerate weed seed predation (Petit *et al.*, 2017).

However, determining the effect of seed predators on weed demography is still a scientific obstacle (Menalled *et al.*, 2000; Petit *et al.*, 2018). Given that seed predators respond differently to weed seed densities, weed patch can be limited, persist and expand. Carabid beetles respond in a density-independent manner or even in an inversely density-dependent one, whereas rodents mainly respond in a positive density-dependent way (Westerman *et al.*, 2008; Baraibar *et al.*, 2012). If seed predators response is inversely density dependent, a higher proportion of seeds is consumed in populations with a lower seed density. In the case where seed predators respond in a direct density-dependent manner, higher proportions of seeds are consumed in weed populations with a larger density. Thus, weed populations are limited if seed predator response is directly density dependent. However, it is still unclear if weed species compensate for the seed losses, resulting in a maintaining and increasing seedbank. Of note, compensation for interventions in the early life cycle stages of the weed *Striga harmonthica* (Del.) Benth. in sorghum have been reported by Westerman *et al.* (2007), which did result in a maintained weed seedbank.

1.3 *Echinochloa crus-galli* in maize

Maize cultivation has been booming in Germany since 2012, when maize became an energy crop for biogas plants. Today, maize is the second most important crop after wheat (Destatis, 2019). As a consequence, the acreage of maize increased by introducing the crop to new arable fields and crop sequences. Weed control in maize mainly targets weed seedlings using

herbicides during the critical period of maize (2–8 leaf stages). Introducing maize to a new field influences the weed species community. Especially, the summer-annual weed species *Echinochloa crus-galli* (L.) Beauv. profits from maize cultivation (De Mol *et al.*, 2015; Pannwitt *et al.*, 2018) in dense maize cropping patterns (Redwitz & Gerowitt, 2018) and causes crop yield losses (Oerke, 2006). Phenotypic plasticity makes it easy for *E. crus-galli* to adapt to local conditions (Maun & Barrett, 1986; Norris, 1996) and complete their life cycle. As genetic variability facilitates *E. crus-galli* evasion of control measures, the evolution of herbicide resistance (Heap, 2019) is the most challenging result of genetic variability. It is a consequence of overusing herbicides on the weed seedlings, in combination with dense maize cropping patterns (Claerhout *et al.*, 2015).

One approach to decreasing the selection pressure for herbicide resistance in *E. crus-galli* is to use a set of diverse management practices targeting different life stages of the weed (Liebman & Gallandt, 1997; Norsworthy *et al.*, 2012). However, prior to the development of effective management practices, we need to understand the complete life cycle of *E. crus-galli.* To date, research on *E. crus-galli* focussed on single stages of the life cycle and its influencing factors (Ogg & Dawson, 1984; Maun & Barrett, 1986; Clay *et al.*, 2005; Norris, 1996; Bagavathiannan & Norsworthy, 2012; Bosnic & Swanton, 1997; Awan & Chauhan, 2016). The summer-annual weed *E. crus-galli* completes its life cycle within one cropping period. It propagates mainly via seeds, as seedlings germinate from a seedbank and grow to adult plants that shed seeds. Germination lasts over the whole vegetative period of maize, with germination occurring only when conditions are most suitable. Crop yield losses are more relevant from early emerging than from late-emerging seedlings (Bosnic & Swanton, 1997; Awan & Chauhan, 2016). With delayed seedling emergence, intra- and interspecific competition increase (Bagavathiannan & Norsworthy, 2012). Competition reduces the number of tillers and panicles per plant (Norris, 1996; Clay *et al.*, 2005), the length and biomass of individual panicles (Maun & Barrett, 1986; Norris, 1996) and, therefore, the level of seed production of *E. crus-galli* (Bosnic & Swanton, 1997). Once seeds are incorporated into the seedbank, they can be viable for 3 to 15 years (Maun & Barrett, 1986), thus buffering the effect of weed control and ensuring the survival of *E. crus-galli* in the long-term. However, to our knowledge, there is a lack of research addressing the interaction of all life stages of *E. crus-galli* in competition with a crop. Such data is needed to parametrize population dynamic models. These models are an efficient and economically feasible tool to evaluate the effect of new IWM measures on long-term population dynamics (Holst *et al.*, 2007; Freckleton & Stephens, 2009).

1.4 Objectives of the study

The goal of this study was to identify the demography of the summer-annual weed species *E. crus-galli*, the effect of seed predation on *E. crus-galli* demography and identify the weed control potential of seed predation. Therefore, this study addresses three obstacles: determine seed predators behaviour, *E. crus-galli* demography and the effect of seed predators on *E. crus-galli* demography. Seven main objectives were tested:

Seed predators behaviour

1. Insect and rodent trapping – identify assemblage of seed predator species in maize fields in north-eastern Germany
2. Response of seed predators to weed seeds
    a. determine density dependence
    b. estimate the level of weed seed consumption
    c. measure the impact of seed exposure time on density dependence and on level of weed seed consumption

Demography of E. crus-galli

3. Optimizing estimation of seed production – testing the relationship of number of seeds and panicle traits
4. Identify the demography of *E. crus-galli*– parameterize the life cycle of *E. crus-galli* population densities

Seed predators effect on the demography of E. crus-galli

5. Target the seed stage – estimate the numerical effect on the subsequent seedling stage
6. Assess whether *E. crus-galli* compensates for seed losses in subsequent life stages
7. Final constant level of seed production – determine the impact of in-field environmental conditions

1.5 Methodology and outline of the study

Two main experimental approaches were used to follow the objectives of the study. Both experiments were located in three intensively managed maize fields in north-eastern Germany that were minimally tilled and has a history of three consecutive years of maize. Soil seedbank that was identified before the start of the experiment and represented a typical weed assemblage in maize, but surprisingly without the presence of *E. crus-galli* (Table 1).

Table 1 Number of seeds m^{-2} weed $species^{-1}$ in soil samples (0-5 cm) randomly taken in 6 plots (n=40 $plot^{-1}$ a 1 inch) prior to the start of the short- and long-term experiment.

Weed species	**Field 1**	**Field 2**	**Field 3**
Anchusa arvensis (L.) BIEB.	345	0	0
Arenaria serpyllifolia L.	99	0	0
*Betula spec.*L.	0	0	148
Chenopodium album L.	10409	5427	5279
Geranium pussilum BURM.	197	0	0
Lamium purpureum L.	0	0	49,3
Tripleurospermum maritimum (L.) KOCH	345	0	0
Papaver rhoeas L.	395	0	0
Polygonum aviculare L.	49	99	345
Fallopia convolvulus (L.) A.LÖVE	2319	0	0
Setaria viridis (L.) P.BEAUV.	99	0	0
Solanum nigrum L.	99	0	0
Spergula arvensis L.	2072	0	0
Stellaria media (L.) VILL.	247	11643	493
Veronica hederifolia L.	49	0	987
Viola arvensis MURR.	5624	0	5525

The study region has a slightly sloping terrain with loamy to sandy soils. Fields were intensively managed and mainly used for the production of wheat, oilseed rape, barley, rye, sugar beet and maize. Experimental fields were embedded in a different landscape matrix; within a 5-km radius, field 1 (12 ha, sandy soil) was surrounded by woodlands and crop fields, field 2 (10 ha, loamy sandy soil) by grassland and maize fields, and field 3 (7 ha, sandy loam) was among woodlands and a highway. We consider field 1 and field 2 to have the highest and lowest landscape complexity, respectively.

Short-term experiment - design

A short-term experiment was conducted to estimate the level of seed predation in response to seed densities of *E. crus-galli*. We conducted the short-term experiment in late summer of two consecutive years, which is the time of natural seed shed of *E. crus-galli* and when seeds are available for seed predators. Seed predation rates were estimated by assessing the number of seeds that remained in the soil of the exposure areas (seed trays) after seed predation (Table 2).

Table 2 Field work activities and duration (coloured gray) of long-term (ltE) and short-term experiment (stE) (indicated by x) and crop management in relation to the development of the maize crop (BBCH) in two subsequent years.

Year	BBCH	Crop Management	Activities in Experiments	ltE	stE
2014			Soil sampling of ambient seedbank	x	x
	63		Experimental set-up installation	x	x
			Echinochloa crus-galli sowing	x	x
			Seed predators trapped	x	x
	89	Maize harvest	Experimental set-up removed		x
			Seedlings rated	x	
			Soil samples further processed (in lab)	x	x
			Carabid beetles identified (in lab)	x	x
2015			Soil sampling spring seedbank & long-term seed predation	x	
		Glyphosate application			
		Mulching around experimental plots			
	0	Crop sowing			
	1		Seedlings rated every second week	x	
	13	Fertilizer application	Herbicide application 1st weed cohort	x	
	16		Herbicide application 2nd weed cohort	x	
			Panicles wrapped	x	
	63		Experimental set-up installation		x
			Echinochloa crus-galli sowing		x
			Seed predators trapped	x	x
	89	Crop harvest	Adult plants and panicles harvested	x	
			Experimental set-up removed	x	x
			Panicle further processed (in lab)	x	
			Soil samples further processed (in lab)	x	x
			Carabid beetles identified (in lab)	x	x

In late summer, the activity-density of seed predators (rodents and carabid beetles) were identified in both experiments as flanking measures. Rodents were trapped using Sherman live traps, and trapping continued until the recapture rate exceeded 50%. Rodents were identified, weighed, sexed, ear tagged and released. Carabid beetles were trapped using pitfall traps during one week and identified to species level.

Long-term experiment – design

The long-term experiment (Figure 1 & Table 2).

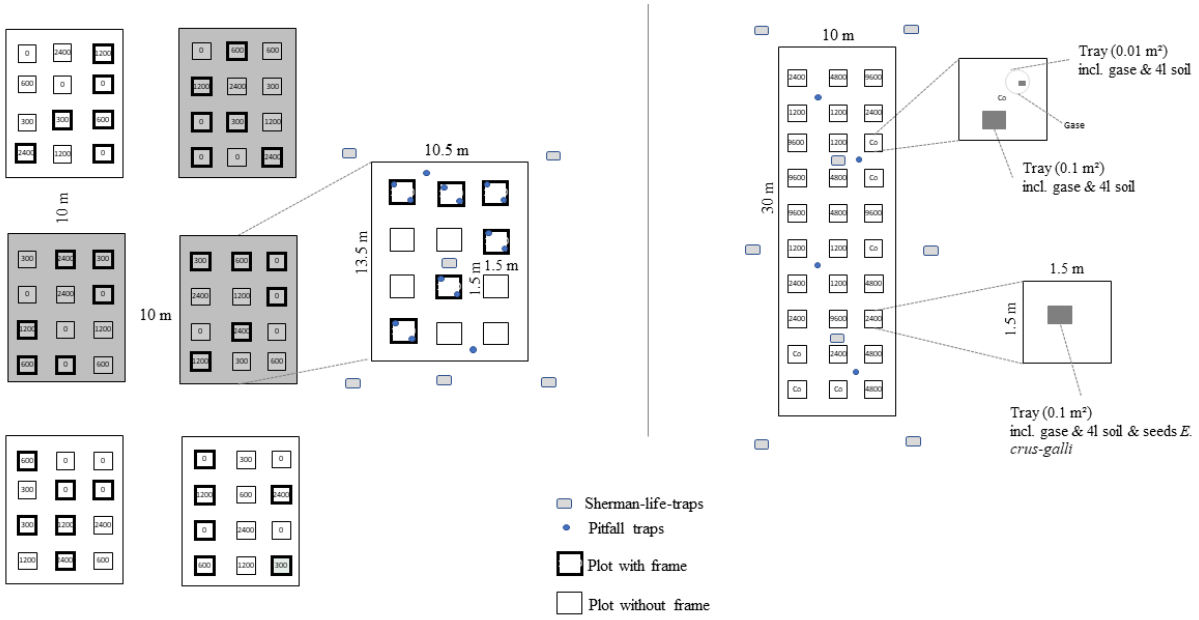

Figure 1 Experimental set-up for the long-term experiment (left) and 2 (right) gray-coloured blocks in the long-term experiment indicate factor intraspecific competition initiated by two herbicide applications and hand weeding; white blocks indicate interspecific competition with other weeds, initiated by one herbicide application.

We followed the life cycle of *E. crus-galli* from seeds to seedlings, adult plants that produced panicles and seed production (+/-), seed predation and (+/-) interspecific competition with a typical weed assemblage in maize. Seed predators were excluded by 0.6-m-high plastic frames that were installed during the length of the experiment. Interspecific competition with other weeds was adjusted by allowing the growth of the second weed cohort. In all blocks, weeds of the first cohort (except for *E. crus-galli*) were controlled by applying herbicides that did not affect *E. crus-galli.* Intraspecific competition was adjusted in half of the blocks by using herbicides applied on the first and the second cohort and hand weeding to control late-emerging weed species.

1.6 Chapter outline

The demography of the weed species *E. crus-galli*, the effect of seed predation on *E. crus-galli* demography and the weed control potential of seed predation in maize fields are considered in Chapters 2–4.

In Chapter 2, the response of seed predators to populations of different densities and times of seed exposure (short- and long-term) in maize crops is examined. Seed predators, i.e. carabid beetles and rodents, were trapped during one week in late summer in the fields using pitfall and Sherman life traps. Data from experiment 2 were used to estimate the response of seed predators on the short-term exposure of seeds. Soil samples of the spring seedbank in

experiment 1 were used to estimate the seeds that are left in the soil after long-term exposure to seed predators. Data were analysed using generalized linear models with a binomial distribution and a logit-link function.

Chapter 3 is an intermediate step, which was necessary to continue to test the hypothesis in Chapter 4. The aim was to simplify the estimation of *E. crus-galli* seed production, testing the relation between panicle traits (dry weight or length) and the number of seeds. We used a dataset representing an aliquot of all harvested panicles (178 from a total of 6491 panicles) from experiment 1. Linear regression models after log-transformed response variables represented the best fit model for analysing factors influencing panicle traits, such as time of seedling emergence, density of *E. crus-galli*, control intensity of other weeds, seed predation and field. Given that the relationship between panicle trait and number of seeds was accurate and precise, this relationship was used to estimate seed production of the remaining panicles. Seed production of all panicles will be used to estimate life cycle stage seed production per unit area in Chapter 4.

Thus, in Chapter 4, we take a closer look at the life cycle of the typical weed species in maize, *E. crus-galli*. Within one full life cycle, we follow the fate of *E. crus-galli* when seeds are targeted or non-targeted by seed predators. Populations of *E. crus-galli* differ in their initial seedbank density and are fully parameterize for all typical life cycle stages (seedbank, seedlings, adult plants, seed production). To clarify whether seed predation is able to control weeds or if the weeds themselves can compensate for the losses, empirical data from the long-term experiment were used. Furthermore, we take a closer look as to whether life stages are density dependent and how stages relate to each other. Linear regression models after log-transformed response variables were used to test the response of each life cycle stage to different densities and seed predation.

Finally, in Chapter 5, the results of each of the three chapters (2–4) will be jointly discussed in a broader context.

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Chapter 2

Biological control of weed patches by seed predators; responses to seed density and exposure time

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Biological control of weed patches by seed predators; responses to seed density and exposure time

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2.1 Summary

Weeds typically occur in patches of different sizes and densities. Post-dispersal seed predators contribute to biological weed control by removing newly produced weed seeds before they can enter the seedbank. Seed predation can limit weed population growth if seed predators respond to seed densities so that seed predation is highest within weed patches. To determine whether this relationship holds for *Echinochloa crus-galli* seeds in corn fields, we observed the levels and responses of seed predators to different densities of *E. crus-galli* seeds during autumn in 2014 and 2015 (August until corn harvest in September - October) and during the winter of 2014 (August 2014 until March 2015) in three corn fields. We tested whether seed predation in autumn is a good estimate of seed predation from seed shed until the following spring (autumn plus winter), because seed predators were assumed to be less active during winter. In autumn 2014, the levels of seed predation and the response to seed density differed between fields because the main type of seed predator varied between fields. In autumn 2015, almost all seeds were lost due to seed predation in all fields, probably because of a high abundance of rodents. A response to seed density could not be detected. Seed predation during winter resulted partially in an increased level and a density independent response to seed density in all fields. Seed predation in autumn does not reflect seed predation from seed shed until the following spring. Furthermore, the effect of seed predation on weed population dynamics will differ between years and fields.

Keywords: Carabid beetle, Corn, *Echinochloa crus-galli*, Population dynamics, Rodent, Weed management

2.2 Introduction

Sensitivity analyses of weed population models have shown that seed mortality, such as caused by seed predators, is one of the most influential life-cycle parameters and changes in this parameter have a disproportionate effect on weed population size (Gonzalez-Andujar & Fernandez-Quintanilla, 2004). Post-dispersal seed predators can contribute to weed control by removing newly produced weed seeds from the soil surface and thus reduce accumulations of seeds in seedbanks in the soil (Westerman *et al.*, 2003; Gallandt, 2006) thereby playing a substantial role in weed management (Davis, 2006).

Because weeds in arable fields typically occur in patches that differ in size and density (Johnson *et al.*, 1996), seed predators need to respond to spatial variability in weed density. The largest impact on weed population density can be obtained if seed predators respond in a direct density-dependent manner; i.e., an increasing proportion of seed predation with increasing seed density (Baraibar *et al.*, 2012). When seed predators respond in an inverse density-dependent manner, i.e., a decreasing proportion of seed predation with increasing densities or in a density independent manner, i.e., a constant proportion of seed predation irrespective of the seed density, weed seeds have a higher or constant probability to survive if they land in a high density weed patch. In these cases, weed populations inside high density patches will increase at greater rates than populations in low density patches with an escalation in the overall abundance of the weed (Westerman *et al.*, 2008).

In the northern hemisphere, granivorous ground beetles, such as *Harpalus* spp. Latreille, crickets, such as *Gryllus pennsylvanicus* Burmeister, harvester ants, such as *Messor barbarus* L. (Baraibar *et al.*, 2011) and granivorous rodents, such as *Apodemus sylvaticus* L., are the main post-dispersal seed predators (Brust & House, 1988; Honek *et al.*, 2003; Baraibar *et al.*, 2012). When granivorous rodents (Hulme, 1993; Marino *et al.*, 2005; Westerman *et al.*, 2008; Baraibar *et al.*, 2012; Daedlow *et al.*, 2014), or harvester ants (Baraibar *et al.*, 2011) are involved, seed predation is generally high and the response to density is usually direct density-dependent. In contrast, when invertebrates, such as carabid beetles or crickets are the main seed predator, seed predation is generally lower (Hulme, 1998) and the response is usually density independent or inverse density-dependent (Zhang *et al.*, 1998; Westerman *et al.*, 2008; Baraibar *et al.*, 2012). The different response to seed densities between granivorous rodents and invertebrates can be a result of differences in metabolism, action radius and the ability to cache seeds. Rodents are homoeothermic and highly mobile with home range of up to several 100 m^2 (Corp *et al.*, 1997) and cache seeds for later consumption (Vander Wall, 1990). Because

rodents are active throughout the year they require food even when no seeds are available. They are able to quickly locate and exploit new weed patches (Daedlow *et al.*, 2014). In contrast, ground beetles are poikilothermic and hibernate in winter. They have an action radius of tens of meters per night (Thomas *et al.*, 1998) and usually do not cache seeds (but see Kirk, 1972). The response depends on patch size (Torra *et al.*, 2016), because invertebrates need more time to locate and exploit new weed patches and do so at a much smaller spatial scale than rodents do (Harrison *et al.*, 2003; Westerman *et al.*, 2008).

One goal of this study was to determine whether seed predation of *Echinochloa crus-galli* (L.) P. Beauv was density-dependent and to estimate levels of seed predation by naturally occurring seed predators in corn fields in north-eastern Germany. In this area, the dominant seed predators are granivorous carabid beetles (Baraibar *et al.*, 2012). Therefore, we expected a relatively low level of seed predation and an inverse density-dependent or density independent response. A second goal of this study was to determine whether measurements on the level of seed predation and the response to seed density made over a short period immediately after seed shed (August – October) are representative of the entire period between seed shed and the following spring. We expected this to be the case, because beetles are not as active under the unfavorable weather conditions experienced in winter. This means that the bulk of seeds will be consumed in autumn. Nevertheless, it is possible that rodents remain active during the winter months. Rodents may elicit a direct density-dependent response and higher levels of seed predation on any seeds left on the soil surface during winter (Harrison *et al.*, 2003; Williams *et al.*, 2009). To determine the level of seed predation that occurred during winter, we compared two different response periods, namely one based on so-called 'seed frames' (Baraibar *et al.*, 2012) that ended with crop harvest (short-term seed predation) and the other that relied on seeding plots and soil sampling which ended in the following spring before seeding of the next crop (long-term seed predation).

2.3 Materials and Methods

Location

Fields were located in Mecklenburg-West Pomerania, a state in north-eastern Germany. The states topography is characterized by a slightly sloping terrain with soil types ranging from loamy to sandy soils. The area is under intensive arable land use and includes the production of wheat, oilseed rape, corn, barley and rye. The average farm size is 284 ha (Statistisches Amt, 2014). During the period of the experiment, temperatures were relatively high as the mean monthly air temperature from August 2014 through March 2015 was 16.6, 15.4, 12.1, 6.8, 2.6,

2.8, 1.5 and 5.3 °C respectively, while the long-term mean (1981-2010) was 17.0, 13.8, 9.3, 4.6, 1.5, 0.6, 1.0 and 3.8 °C respectively. From August until October 2015, mean monthly air temperatures were 19.4, 13.6 and 8.5 °C (German Meterological Service, 2016).

Weed seed predation study sites

In 2014 and 2015, the proportion of seed predation was measured on three commercial fields that had been under continuous corn for at least three years and were managed with minimal tillage. Field 1 (11.8 ha, N 53° 33′, E 11° 08′, sandy soil) was bordered by forest and crop fields (2014, rapeseed; 2015, barley), field 2 (10.1 ha, N 53° 97′, E 11° 98′, loamy sandy soil) was bordered by grassland and crop fields (2014, wheat; 2015, corn and rye) and field 3 (7.0 ha, N 54° 02′, E 12° 02′, sandy loam) was bordered by forest and a highway. On all three study sites and years, corn was sown between 17 April and 7 May. The fields were used both for examining the long-term and the short-term seed predation. Field management, such as pre- and post-emergence herbicide application, the date of harvest and tillage after harvest, differed between study sites (Table A.1).

Selected model weed species

Echinochloa crus-galli was selected as the model weed species in these experiments. It is one of the most important weed species in corn in Germany (De Mol *et al.*, 2015) and palatable to most post-dispersal seed predators (Cromar *et al.*, 1999). Furthermore, soil sampling of the seedbank before sowing, had indicated that no or negligible numbers of *E. crus-galli* seeds were present in the fields. For both experiments, *E. crus-galli* seeds were provided by Appels Wilde Samen GmbH (Darmstadt, Germany). Two seed lots were used, one harvested in 2013 was used in the short-term experiment and the other harvested in 2014 in the long-term experiment. The 1000 seed weight was determined by manually counting seeds (n = 8) and weighing them. The 1000 seed weight was 2.25 g for the first seed lot and 1.38 g for the second seed lot. Prior to seeding, the seeds for the short-term experiment were boiled for at least 5 min to avoid germination. This procedure does not affect palatability to seed predators (Baraibar *et al.*, 2012). For the long-term experiment, seeds were not killed to be able to count and follow the fate of the seedlings over summer; an objective that falls outside the scope of this study.

Short-term seed predation - experimental design

Short-term seed predation was measured in all three fields from August until corn harvest in September - October in 2014 and 2015 (Table 3). The experiment was organized as a complete

randomized block design with three blocks (fields 1, 2 and 3), and five seed densities of *E. crus-galli* (0, 1200, 2400, 4800 and 9600 seeds m^{-2}).

Table 3 Seeding and sampling date of *E. crus-galli* seeds on the three maize fields (field 1,2,3) in the short- (autumn) and long-term (overwinter) experiment 2014 and 2015 in north-eastern Germany.

Site	Year	Experiment	Seeding date	Sampling date
Field 1	2014	short-term	21 August	1 October
	2014	long-term	21 August	17 March
	2015	short-term	20 August	6 October
Field 2	2014	short-term	20 August	30 September
	2014	long-term	20 August	18 March
	2015	short-term	18 August	5 October
Field 3	2014	short-term	20 August	19 September
	2014	long-term	20 August	19 March
	2015	short-term	19 August	2 October

There were six replicates per seed density and the whole experiment was repeated in time (2014 and 2015). Per field, 30 plots (1.5 x 1.5 m) were stacked out at a distance of 1.5 m from each other and at least 50 m away from the field edge. Seed frames, 0.1 m^2, were used to measure the proportion of seed losses due to seed predation. With seed frames, the response of seed predators can be measured over longer periods and towards higher seed densities (Westerman *et al.*, 2008; Baraibar *et al.*, 2012; Davis *et al.*, 2013) than, for example, with seed cards (Westerman *et al.*, 2003). Furthermore, the substrate is more natural; as in the field, seeds can become incorporated into the soil matrix, thus limiting their availability to seed predators, but without losing seeds to deeper soil layers. The frames were constructed of 10 cm high strips of stainless steel that were bent into squares of 25 x 40 cm, lined with fine cloth and filled with seed-free soil that was excavated from below the plough-layer in each field. One frame was set randomly between the crop plants of each 1.5 x 1.5 m plot and buried into the soil such that a 0.5 cm rim was left above the soil surface to ensure the seeds did not wash or blow out of the frames (Baraibar *et al.*, 2012; Daedlow *et al.*, 2014).

Both plots and frames received a predetermined amount of seeds. The required number of seeds for use in the seed frames was counted manually, while the required number of seeds for the area around the trays was weighed based on the 1000 seed weight. All seeds were applied manually. To mimic the normal period of seed shed of *E. crus-galli*, sowing was done in August. At the end of the season, shortly before corn harvest, the seed frames were removed

and soil from the frames was stored at 4 °C until further processing (Sub-section ´Long-term seed predation – experimental design`).

Control frames

In the short-term seed predation experiment, each of six randomly chosen plots per field were equipped with both a positive and negative control in the same plot. Positive controls served to measure seed losses due to other causes than seed predation. Positive control seed frames (2014, 10 x 10 x 5 cm; 2015, 25 x 40 x 5 cm) were filled with seed free soil and seeded with 100 seeds of *E. crus-galli.* They were surrounded by a 0.6 m high, fine-meshed plastic screen, buried at a depth of 5 cm in the soil, to prevent access to seed predators. Negative controls served to measure natural seed additions, mainly due to seed shed. They also consisted of a seed frame (25 x 40 x 5 cm) filled with seed-free soil, but no seeds were applied and the frames were not protected by a screen.

Long-term seed predation - experimental design

Long-term seed predation was estimated in an experiment adjacent to the short-term experiment in the same fields from August 2014 until March 2015 (Table 1). In each field, six blocks of 10.5 x 13.5 m, at least 8 m apart, contained 12 plots of 1.5 x 1.5 m, at least 1.5 m apart. The experiment was installed as a complete randomized block design. *Echinochloa crus-galli* seeds were applied at 300, 600, 1200, 2400 seeds m^{-2} in two plots per block, while four plots served as controls (no seeds). Seed densities used here were lower than in the short-term experiment, because we needed to be able to count seedlings in order to follow the fate of the seeds over summer. As said before, that objective falls outside the scope of this study. As soon as seeds were obtained from the supplier, they were manually applied in August 2014. Only after seeding, the viability was tested via a TTC-Test (n = 591; (Moore, 1985)). Because the viability was relatively low 63%, we would not be able to reach the pre-determined seed densities. To correct this error, additional seeds were applied one week after the first sowing. For this purpose, non-viable seeds were removed from the seed lot using a seed cleaner (The Real Seed Collection Ltd, 2016), which increased the viability to 89% (n = 353) and the 1000 seed weight to 1.54 g. Seed predators were excluded from half the plots using 60 cm high plastic exclusions (Polyethylene 300, Buck & Sohn Kunststoffe, Hamburg, Germany).

In March 2015, we took soil samples to estimate the proportion of long-term predation. To determine the density of seeds remaining after winter, 40 soil samples of the top 5 cm were taken with an auger (Ø 2.54 cm) in the outer 50 cm of each 1.5 x 1.5 m plot. Soil samples were

stored at 4 °C until further processing. Seeds were retrieved from the soil in three steps. First, the organic matter and larger particles were separated from the rest of the soil via elutriation (Elutriator E48X; Disema, Bellvis, Spain; (Wiles *et al.*, 1996)). Next, large sandy particles were removed via a flotation technique using a saturated salt solution. The remaining organic material was subsequently rinsed and dried at 60 °C. Finally, the number of intact seeds was counted under a binocular microscope.

Identification of seed predators

Carabid beetles were trapped during one week in late August of 2014 and 2015. Per field, 18 pitfall traps were distributed over the area occupied by both experiments. Pitfall traps consisted of two stacked, rectangular (10 x 7.5 x 10 cm) plastic cups, buried flush with the soil surface and filled with a mixture of water, 10% ethylene glycol and some Tween®20. The catch was stored in 70% ethanol at 4 °C until identification. Samples were identified to species level using (Dücker *et al.*, 1997), counted and numbers were pooled per field and year.

Rodents were trapped using 45 Sherman live traps per field distributed over the area occupied by the two experiments. Trapping was done around new moon when rodent activity is supposed to be highest (Plesner Jensen & Honess, 1995). Traps were baited with small balls made of wheat flour, peanut butter, oat, oil and water. Synthetic bedding material was added to the trap. Two nights before trapping started, traps were left open to allow rodents getting accustomed. Sherman traps were set and checked every morning. Trapping continued until the percentage of recaptures exceeded 50%, for a minimum of three consecutive nights, namely from 20 to 29 August in 2014 and from 8 to 17 September in 2015. Trapped rodents were identified by species and sex. They were weighed, marked individually by an ear-tag and released. The total number of rodents caught was summed over traps and nights (trap nights) and pooled per field and year.

Data analysis

The effects of field (1, 2 and 3), year (2014, 2015), seed density (1200, 2400, 4800 and 9600 seeds m^{-2}) and the interactions between main effects on short-term seed predation, Q_S were tested by a generalized linear regression model with a binomial distribution, and a logit-link function. Q_S, was calculated as

$$Q_S = \frac{S_i - S_r}{S_i} \qquad [m^{-2}] \qquad (1)$$

with S_i, the number of seeds applied, and S_r, the number of seeds remaining after short-term exposure to seed predators. In 2014, damage of seed frames by wild boars required the

exclusion of one data point in field 2 and 16 in field 3. In 2015, three data points of field 3 were excluded for the same reason.

Preliminary analyses had indicated a significant interaction between field and year ($P = 0.0001$) and, therefore, as a next step, the analyses were repeated for each year separately. A significant interaction between seed density and field in 2014 ($P = 0.0012$) prompted a separate analysis of the effect of seed density on Q_S per field for 2014. To be able to compare the results between years, data for 2015 were also analyzed per field.

Q_L, the long-term seed predation, was calculated as the difference between seed losses from plots without exclusion ($Q_L + M$) and seed losses from plots where seed predators were excluded (M). As a first step, the seedbank mortality, M, was estimated. Seedbank mortality appeared to be very low and occasionally more seeds were recovered from plot with exclusions (S_{re}) than initially seeded (S_i), which resulted in estimates of $S_{re} / S_i \geq 1$. To avoid this, these data were analyzed using the proportion of recovered seeds, $C = S_{re} / S_i$, which appeared to be approximately normally distributed. The effects of field (1, 2 and 3), seed density (300, 600, 1200 and 2400 seeds m^{-2}), and the interaction between field and seed density on C were tested in a linear regression model. If C is density independent, we expect that C is constant for all densities. If C is density-dependent, we expect a significant effect of seed density. C appeared density independent and also the effect of fields was insignificant. This resulted in a single mean value for all fields and all seed densities of $C = 0.988 \pm 0.384$. Therefore, M was estimated as $M = 1 - 0.988 = 0.012$.

Next, long-term seed predation, Q_L, was calculated as

$$Q_L = \frac{S_i - (S_{rn} + M)}{S_i} \qquad [m^{-2}] \qquad (2)$$

where S_{rn}, is the number of recovered seeds from plots without exclusion. In four out of the 72 plots Q_L was smaller than zero. In these cases Q_L was assumed to be zero. The effects of field and seed density and their interaction on Q_L were tested using a GLM with a binomial distribution, and a logit-link function.

To test whether the level of seed predation (intercept of the regression lines) and the response to seed density (slope of the regression lines) were similar for the short-term and long-term seed predation trials, a GLM was constructed to analyze the combined data set. Data of short-term and long-term trials were compared for 2014 only, because the long-term experiment was not repeated in 2015. Models were constructed per field and contained trial duration (short-term, long-term), seed density and the interaction between field and trial duration as explanatory variables. All analyses were performed in R version 3.1.2 (R Core

Team, 2014), using the package "nortest" (Gross & Ligges, 2015) to test for the normality of models residuals, package "faraway" (Faraway, 2014) to create the inverse logit function and package "lsmeans" (Lenth, 2015) to compare intercept and slope of the regression lines of the short- and long-term seed predation experiments.

2.4 Results

Controls

Positive controls to check for seeds lost due to abiotic factors instead of seed predation failed their function, because seeds recovered from frames showed signs of feeding marks by carabid beetles. Apparently, carabids were able to fly over or crawl into the structures, causing additional seed loss. It is also possible that some carabids were trapped inside the structure during installation. Consequently, the number of recovered seeds was low (40 – 85%). Therefore, data from the positive controls were omitted from the analysis.

Negative controls to check for seed additions from outside the plots of the short- and long-term trial due to seed shed, rain splash, wind, etc. indicated that very few seeds (0 – 1 seed per frame) entered the plots, and the effect was considered negligible.

Short-term seed predation

In 2014, the effect of seed density was non-significant ($\chi^2 = 0.193$, df = 22, $P = 0.116$) in field 1, indicating density independence, significant ($\chi^2 = 1.209$, df = 22, $P < 0.0001$) in field 2, indicating inverse density-dependence, and significant ($\chi^2 = 0.669$, df = 6, $P = 0.0485$) in field 3, indicated direct density-dependence. The percentage of explained deviance of the data was 10% for field 1, 38% for field 2 and 36% for field 3.

In 2015, much higher proportions (0.876 ± 0.017) of seeds were lost than in 2014 (0.380 ± 0.028) (Figure 2). These high proportions, disguised any response that seed predators might have had to increasing seed density. The GLM with an explained deviance of 59% indicated a significant effect of field only ($\chi^2 = 6.4207$, df = 66, $P = 0.0001$), suggesting that the level of seed predation differed between fields.

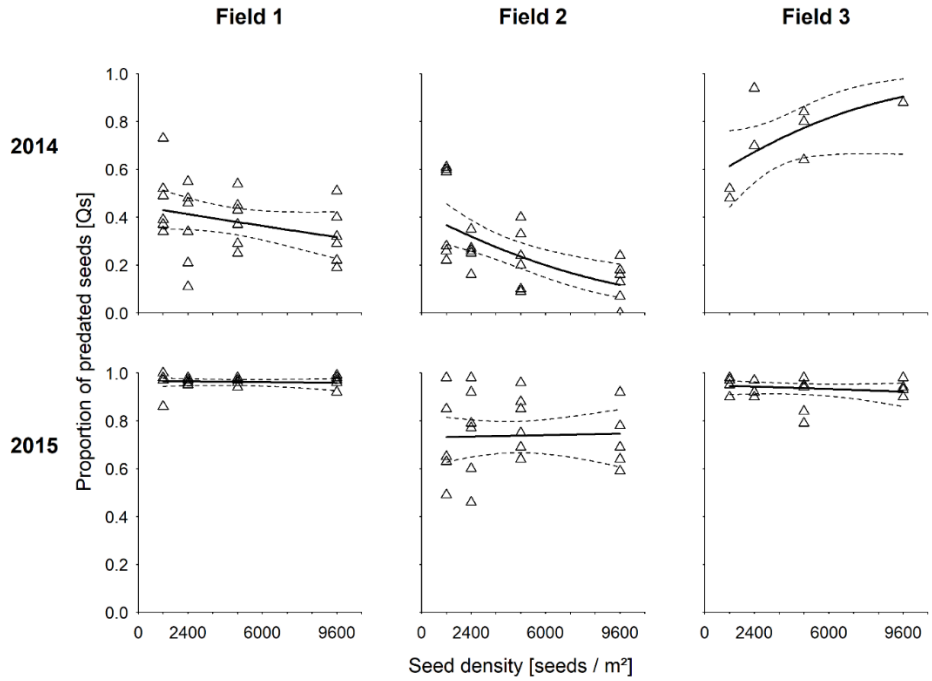

Figure 2 The relationship between the proportions of seeds lost from the seed frames in fields 1, 2 and 3 between August and corn harvest in 2014 and 2015 and the seed density applied. Points indicate observations, solid lines represent regression lines and dashed lines the confidence interval (95%) around the regression lines.

Long-term seed predation

The proportion of seeds lost from the seedbank between August 2014 and March 2015 was significantly influenced by field ($\chi^2 = 5.254$, df = 69, $P < 0.0001$) and seed density ($\chi^2 = 1.081$, df = 68, $P = 0.04$). The model explained 23% of the residual deviance. Analyses done for each field separately, indicated a density independent seed loss in all fields (field 1, $\chi^2 = 0.678$, df = 22, $P = 0.15$; field 2, $\chi^2 = 0.156$, df = 22, $P = 0.51$; field 3, $\chi^2 = 0.340$, df = 22, $P = 0.12$) (Figure 3). The models explained 7% of the deviance in field 1, 2% in field 2 and 8% in field 3.

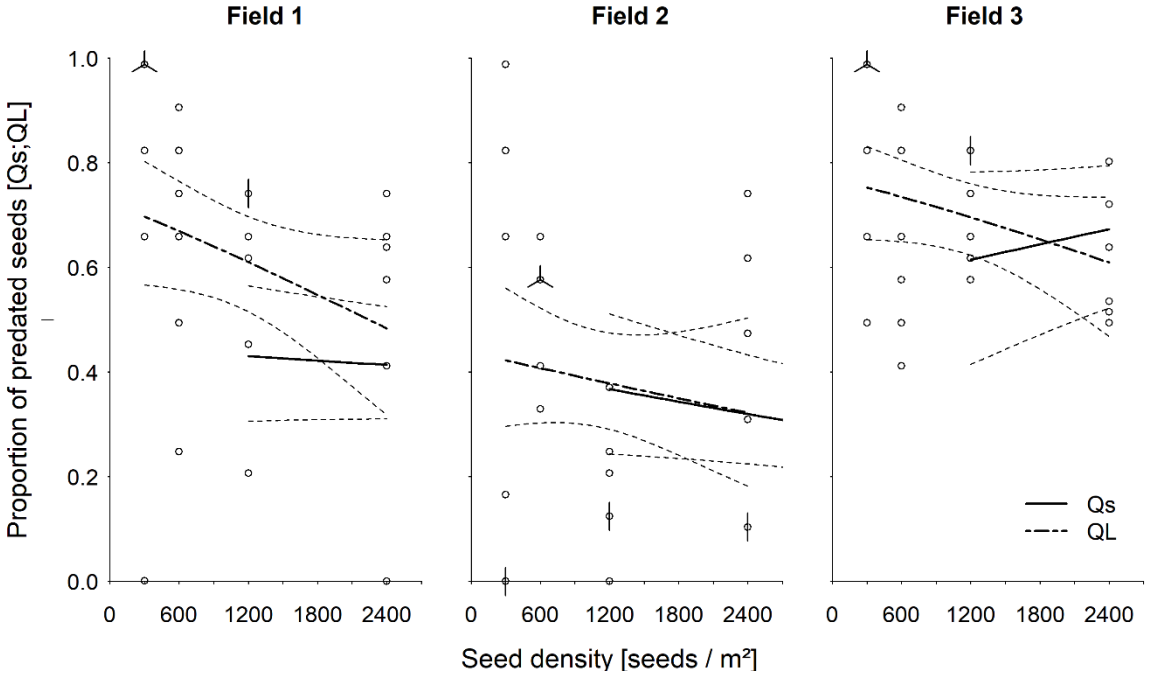

Figure 3 The relationship between the proportion of seeds lost between short-term (Q_S) and long-term (Q_L) and seed density applied. Points indicate observations from long-term predation and lines around points the number of identical values, solid lines represent regression lines and dashed lines the confidence interval (95%) around the regression lines in field 1, 2 and 3.

Comparing short-term with long-term seed predation

Results of the level (intercept) and response to density (slope) between short- and long-term seed predation varied between fields. The level of seed predation was significantly different in field 1 and did not differ in fields 2 and 3. The response of seed predators to densities significantly differed in field 3, with a direct density-dependent response during the short-term trial and a density independent response during the long-term trial. Seed predation in fields 1 and 2 did not differ in the response to densities between a short or a prolonged exposure time (Table 4).

Table 4 Short- and long-term seed predation per field (1, 2, 3) in the year 2014 - 2015 obtained from results of intercept and slope of best fitted models given on the logit scale with significant differences indicated by *P*-values of Chi-Square-test *P* (χ^2) and number of repeated plots, n.

Site	Experiment	n	Intercept	P(χ^2)	Slope	P(χ^2)
Field 1	short-term	24	-0.21	0.01	-0.58×10^{-4}	0.13
	long-term	24	0.96		-4.30×10^{-4}	
Field 2	short-term	23	-0.33	0.87	-1.80×10^{-4}	0.91
	long-term	24	-0.25		-2.10×10^{-4}	
Field 3	short-term	8	-0.21	0.10	-2.10×10^{-4}	0.03
	long-term	24	1.20		-3.10×10^{-4}	

Identification of seed predators

Carabid beetles were the dominant invertebrates in pitfall traps. Most of these are known to be granivorous. *Harpalus rufipes* DeGeer, *Calathus fuscipes* Goeze and *Calathus melanocephalus* L. were most frequently trapped in all fields. The number of carabid beetles caught varied between years (Table 5).

Table 5 The number of most frequently trapped carabid beetles, in sixteen pitfall traps per field (1, 2, or 3) in a single week in August 2014 and 2015.

Carabid beetles	**Field 1**		**Field 2**		**Field 3**		**Food habits**[1]
	2014	2015	2014	2015	2014	2015	
Amara consularis Duftschmid, 1812	49	79	1	0	0	4	g
Amara fulva O. F. Müller, 1776	82	59	0	0	18	59	g
Calathus erratus Sahlberg, 1827	69	123	1	0	0	0	o
Calathus fuscipes Goeze, 1777	85	221	114	393	109	70	o
Calathus melanocephalus L., 1758	61	101	47	16	17	26	o
Harpalus affinis Schrank, 1781	7	57	1	5	0	5	g
Harpalus calceatus Duftschmid, 1812	1	20	0	0	0	0	g
Harpalus griseus Panzer, 1796	1	48	0	0	0	0	g
Harpalus rufipes DeGeer, 1774	624	233	3	53	8	92	g
Poecilus lepidus Leske, 1785	1	76	0	0	0	0	g
Pterostichus melanarius Illiger, 1778	1	11	30	63	23	14	o
Total rare carabid beetles	60	152	143	170	77	90	g/o
Total carabid beetles	1041	1180	340	700	252	360	g/o

[1] based on (Goldschmidt & Toft, 1997) and (Lundgren, 2009)
g= granivorous; o = omnivorous

The most frequently caught rodent species were *Apodemus agrarius* Pallas and *A. sylvaticus* L. (Table 6). The number of small rodents differed between years and fields. In 2015, a higher absolute number of rodents were trapped in fields 1 and 3 (62 and 69 respectively), compared to 2014 (4 and 11 respectively), especially with regard to *A. agrarius* (1 vs. 72). In field 2, the dominant species was *A. sylvaticus* and was most frequent in 2014 (Table 6).

Table 6 Number of rodent species trapped in field (1, 2, 3) during new moon in August 2014 and September 2015 at a certain number of trap nights (t).

Rodent species	Field 1		Field 2		Field 3	
	2014 (t=135)	2015 (t=135)	2014 (t=180)	2015 (t=135)	2014 (t=180)	2015 (t=180)
Apodemus agrarius Pallas, 1771	0	37	1	1	1	35
Apodemus flavicollis Melchior, 1834	0	0	0	0	2	0
Apodemus sylvaticus L., 1758	3	13	29	20	5	9
Mycrotus arvalis Schrank, 1798	1	12	1	7	3	25

2.5 Discussion

We had expected a low level of seed predation and an inverse density-dependent or density independent response to seed density. However, this study showed that in autumn the level of seed predation and response of seed predators to seed density varied between fields and years. A high level of seed predation and direct density dependence are usually associated with granivorous rodents (Baraibar *et al.*, 2012), and a lower level of seed predation and inverse density dependence or density independence with invertebrates (Marino *et al.*, 2005; Westerman *et al.*, 2008). By extension, different patterns of seed predation are most likely caused by different seed predators.

In 2015, a high proportion of seed predation (0.49 - 1.00) was found in the short-term experiment in all fields, irrespective of seed density. This means that we were unable to determine whether seed predation was directly or inverse density-dependent. These results are consistent with a much higher population of rodents in 2015 as compared to 2014. Similar results were reported by (Daedlow *et al.*, 2014), who found high seed predation rates and high rodent populations in Spain. A consequence is that in 2015 practically all seeds in the 2.25 m^2 large patches were consumed before corn harvest.

In the previous season (2014), results of the short-term experiment differed between fields. In field 1 in 2014, the response to density was density independent and that at a low level (0.11 - 0.77). Here, a large number of granivorous carabids, in particular *H. rufipes*, were present. This is consistent with our expectations, namely that granivorous carabids cause a density independent response and a lower level of seed predation than granivorous rodents do. In field 3, the response to density was direct density-dependent at a relatively high level of seed predation (0.48 – 0.94). This concurs with the fact that rodents were the main seed predator. In

field 2, inverse density dependence was found at a low levels of seed predation (0 – 0.61). In this particular field and year, the number of rodents (e.g., *A. sylvaticus*) was also high. This should have led to direct density dependence. However, in this particular case, we suspect that the high numbers of rodents was caused by the fact that a large neighboring cereal field (100 ha) had been harvested a few days before the onset of rodent trapping. It is likely that the resident rodent population had left the bare field and passed through the corn field looking for suitable habitat. The inverse density dependence was in that case caused by omnivorous carabid beetles (e.g., *C. fuscipes*) that were also abundant. Consequently, in both fields 1 and 2 seed predation was mainly caused by carabid beetles. However, the activity-density of carabid beetles was three times higher in field 1 than in field 2, while the level of seed predation was only slightly higher in field 1 than in field 2. Several studies have found a correlation between the level of seed predation and the activity-density of carabid beetles (Cromar *et al.*, 1999; Rusch *et al.*, 2016). A similar number of studies have found no correlation at all (Honek *et al.*, 2003; Saska *et al.*, 2008). Whether a correlation was found or not seems to depend on many factors, such as region (Jonason *et al.*, 2013), food habits (Mauchline *et al.*, 2005; Trichard *et al.*, 2013), weed species (Simard *et al.*, 2013) or season (Honek *et al.*, 2003; O'Rourke *et al.*, 2006). A possible explanation for our results could be that in field 1, carabid species were mainly granivorous, such as *H. rufipes,* and in field 2, they were mainly omnivorous, such as *Calathus* spp. and *P. melanarius.* For decades, research has tried to unravel the factors responsible for different species composition and activity-density of carabid beetles. Multiple abiotic (Holland & Luff, 2000) and biotic factors (Kulkarni *et al.*, 2015) were found, however, we do not know, which factor was decisive in our study. Furthermore, it is much less clear what factors influence the abundance of granivorous rodents (but see Heroldová *et al.*, 2007; Fischer *et al.*, 2011).

Given the variability in the level of seed predation and the response to density, the effect of seed predators on weed population dynamics will vary between fields and years. In some fields and years, weed patches may grow because the level of seed predation was relatively low and seed predators responded poorly to locally increased seed densities. In other fields and years, weed patches may decrease in size and density because the level of seed predation was high and weed patches had most seed predation.

The estimates on the level of seed predation overwinter concur with results from other studies (Harrison *et al.*, 2003; Williams *et al.*, 2009; Davis *et al.*, 2013). We had expected that seed predation in autumn would be representative of seed predation over the entire winter; because carabid beetles are the main seed predators and they are not active over the winter.

With prolonged exposure, the response to seed density had become density independent in all fields and the level of seed predation increased in one out of three fields. Apparently, seed predators did consume seeds during winter.

In field 1 only, the level of seed predation increased between autumn and spring because the response to density was already density independent. There are several explanations for why the level of seed predation in field 1 increased overwinter. In this field, carabid beetles, mainly *H. rufipes*, were active. Carabids are active when temperatures are above 8 – 10 °C (Honek, 1997; Saska *et al.*, 2010). In the winter of 2014, temperatures were above 8 °C on 48 days between October and March. In normal years, this happens only on 30 days (based on mean values of daily temperature 2009 – 2015; (German Meterological Service, 2016). Thus, the higher level of seed predation could be the result of a longer period of activity of carabid beetles. Alternatively, the increase in the level of seed predation overwinter may have been caused by seed predators that were not monitored, such as surface-feeding earthworms or birds. Earthworms are known to collect and cache seeds in burrow systems, also during warm and wet winter (Schutte *et al.*, 2010), such as was the case in 2014 - 2015. We observed earthworm activity in field 1. Earthworms were excluded from the short-term experiment because the seed frames were lined with a fine cloth which prevented access to earthworms. In contrast, in the long-term experiment, earthworms did have access to the seeds. Furthermore, in autumn, we observed bird activity by the presence of bird droppings and prints, but very few birds were actually seen in the corn field. However, granivorous birds may have entered the field after crop harvest.

In field 3, the response of seed predators to seed density changed from direct density-dependence in autumn to density independence in spring. A similar result had been found for seed predation in autumn in corn fields in Iowa, USA, involving field crickets and carabid beetles as seed predators (Westerman *et al.*, 2008). There, density independence after prolonged exposure was explained by the extra time that invertebrates needed to detect and respond to seed patches. We did not determine seed predator populations in winter, but in autumn the populations of rodents and carabid beetles in field 3 were low. In the winter of 2014 – 2015, we observed many new entrances to rodent burrow systems, suggesting rodent activity. However, in the case of rodent activity, a direct rather than an inverse density-dependent response would have been more likely (see introduction).

Field 2 was the exception, as neither the level of seed predation nor the inverse density - dependent response to seed density changed between autumn and spring. The carabids present

were mainly omnivorous and not specialized on seeds. The rodent population was transient and not likely to have remained in the corn field after crop harvest. A nearby winter rye field had sufficient canopy cover and would have been a more attractive habitat for rodents than the corn field (Heggenstaller *et al.*, 2006).

In summary, the results of the autumn trial were usually not representative of the fate of seeds during the entire period between seed shed and the following spring. Based on the density independent response, seeds inside and outside patches have the same probability of being consumed by seed predators. The only factor influencing weed population dynamics is the level of seed predation. Unfortunately, seed predation in the autumn of 2015 could not be compared to seed predation overwinter in 2015, because no long-term seed predation trial was initiated in 2015. However, in 2015, almost all seeds were consumed by seed predators by the end of the autumn trial anyway. Therefore, in 2015, the two estimates would have been similar. It could have led to erroneous conclusion that seed predation in autumn is representative of seed predation over the entire winter season.

We used seed frames in autumn and soil sampling for overwinter measurements. The two methods differed in the variability of the results. Because seed frames contained an exactly known number of seeds and no sampling was involved, the error in the number of recovered seeds was relatively small. Plots used for measuring seed predation via soil sampling were seeded with a weighed number of weed seeds. In addition, soil sampling added substantial sampling error (Grundy, 2003). Although care was taken to distribute seeds as uniformly as possible, subsequent actions by rain and wind may have caused clustering of seeds. Consequently, the results from soil sampling of the seeded plots were less precise than the results from seed frames. However, seed frames cannot be used over the entire winter period. For example, they may be damaged during corn harvest. Seeded plots plus soil sampling is the only method available for the purpose of measuring seed predation over the entire winter.

To control weed patches, a high level of seed predation is needed and a direct density-dependent response will be most favorable. The response we observed in the fields was always density independent and only differed between fields in the level of seed predation. Weed patches may persist but their density will be reduced but by how much will depend on field and year. Given that seed predation occurred almost always at high levels, it has a clear potential to regulate weed patches of *E. crus-galli*. As a next step, we will investigate the joint effect of seed predation with other weed demographic parameters on the population dynamics of *E. crus-galli*.

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Appendix A

Table A.1 Crop management practices on the three fields in the short- (autumn) and long-term (overwinter) experiment 2014 and 2015 in north-eastern Germany.

Site	Year	Experiment	Seeding rate (seeds m^{-2})	Row spacing (cm)	Seeding date	Harvesting date	Tillage
Field 1	2014	short-term	80 000	no rows	24 April	1 October	none
	2014	long-term	80 000	no rows	24 April	2 October	none
	2015	short-term	80 000	75	17 April	7 October	chisel ploughing
Field 2	2014	short-term	86 000	75	5 May	30 September	chisel ploughing
	2014	long-term	86 000	75	6 May	30 September	none
	2015	short-term	86 000	75	7 May	21 October	chisel ploughing
Field 3	2014	short-term	80 000	75	26 April	21 September	chisel ploughing
	2014	long-term	80 000	75	26 April	21 September	none
	2015	short-term	80 000	75	25 April	7 October	none

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Chapter 3

Using panicle dry weight to estimate seed production in *Echinochloa crus-galli*

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Using panicle dry weight to estimate seed production in *Echinochloa crus-galli*

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3.1 Summary

A better understanding of weed seed production is a key element for any long-term management allowing some weeds to shed seeds. The challenge with measuring seed production in weeds is the large effort required in terms of time and labour. For the weed species *Echinochloa crus-galli* it was tested whether the number of seeds per panicle dry weight or per panicle length can be used to estimate seed production. Experiments were conducted in three maize fields in north-eastern Germany. The effect of factors that could influence this relationship, such as the time of seedling emergence, the density of *E. crus-galli*, the control intensity of other weeds, seed predation and field were included. A few days before maize harvest, all panicles were removed, weighed, panicle length was measured, and for a subsample of 178 panicles, the number of seeds was counted manually. Panicle dry weight predicted the number of seeds per panicle better ($R^2 = 0.92$) than did panicle length ($R^2 = 0.69$). The other factors except for "field" and "seed predation" had no effect on these relationships. The relationships between seed number and panicle dry weight found in this study closely resembled the results reported in an earlier study. Based on our results, we emphasize that both plant traits are appropriate to estimate seed production, depending on the users´ demand for precision and available resources for evaluating sustainable weed management strategies.

Keywords: ECHCG, barnyardgrass, maize, fecundity, panicle length, panicle weight

3.2 Introduction

Integrated weed management aims to maintain the control over weeds while at the same time reducing the use of herbicides whenever possible. Within this framework, an important tool is to accept weeds below economic thresholds (Barzman *et al.*, 2015). Consequently, in integrated weed management some weeds may survive and produce seeds at the end of the season. *Echinochloa crus-galli* (L.) Beauv. (barnyardgrass) is a common weed of maize (*Zea mays* L.) (Maun & Barrett, 1986; De Mol *et al.*, 2015; Redwitz & Gerowitt, 2018), the second most important crop in Germany (Destatis, 2018). The effectiveness of chemical control of *E. crus-galli,* the basis of weed control in commercial maize cultivation, is threatened by the high risk of herbicide resistance evolution (Claerhout *et al.*, 2016; Heap, 2018). Seeds shed from weeds that survived control, either planned due to integrated management or unplanned due to resistant biotypes, challenge us to understand the seed production of *E. crus-galli*. The seed production is important if we want to assess weed management methods and concepts in their entirety (Norris, 2007; Norsworthy *et al.*, 2012). Data on seed production is also required in long-term predictions of weed populations via simulation models (Holst *et al.*, 2007; Freckleton & Stephens, 2009), as the one published by Redwitz *et al.* (2016) for *E. crus-galli*.

In weed population models that focus on a single weed species, seed production usually appears to be highly influential on population size (Gonzalez-Andujar & Fernandez-Quintanilla, 2004). Unfortunately, there are serious problems associated with the generalization of the techniques for estimating seed production. Ignoring this problem could lead to unrealistic model outcome. The estimation of seed production in experiments is often limited to conditions of a single field and do not account for the plasticity of weed species caused by intra- and interspecific competition and delayed emergence (Norris, 2007). This applies to *Echinochloa crus-galli*, an example of a plastic weed species that germinates and reproduces throughout the entire maize cropping season (Maun & Barrett, 1986; Norris, 1996). With delayed seedling emergence, intra- and interspecific competition increase (Bagavathiannan *et al.*, 2012). This limits the number of tillers and panicles per plant (Norris, 1992a, 1996; Clay *et al.*, 2005) and length and biomass of individual panicles (Maun & Barrett, 1986; Norris, 1996) and therefore the level of seed production of *E. crus-galli* (Bosnic & Swanton, 1997; Norris, 2007). Therefore, before applying techniques to estimate seed production, proof of concept for the ability to deal with the plasticity of the weed species is needed.

Another complicating factor when measuring seed production is the immediate seed shed of *E. crus-galli* as soon as seeds are ripe. Thus, the timing to measure seed production becomes crucial. Gathering seeds too soon and a certain proportion of the seeds will not be ripe yet, gathering seeds too late and a certain proportion of seeds has been shed already. Both can lead to underestimation of (viable) seed production. A correct estimation of seed production requires a measuring technique that either involves the collection of all seeds during the whole process of seed shed or that is independent of the time of seed shed.

Direct methods to estimate seed production, such as sticky boards or pans, are generally inexact due to seed losses, especially at low seed densities (Norris, 2007). An alternative is counting all seeds per plant or m^2. Because this method is extremely time consuming, researchers have been searching for more efficient methods that are based on the allometry of plants, i.e. the relationship between seed production and individual vegetative or reproductive biomass (Thompson & Stewart, 1981; Weiner *et al.*, 2009), panicle dry weight or panicle length (Norris, 1992b; Forcella *et al.*, 2000).

Norris (1992b) established relationships between seed production and panicle length and panicle dry weight for *E. crus-galli* when the plant was growing in a pure stand. His experiments were conducted in the Mediterranean climate of the Central Valley of California, USA. It is unknown whether the relationships he found are applicable to the conditions in north-eastern Germany, or whether they are applicable for *E. crus-galli* grown under competition with crops or other weed species. For a methodology to be generally applicable, the relationships between seed production and the panicle traits (e.g. panicle length or panicle dry weight) need to be solid under a range of field conditions.

Environmental differences between the two locations, California (USA) and north-eastern Germany, and genetic differences between populations may alter the relationships between seed number and panicle dry weight/ panicle length. Furthermore, the relationships between seed number and panicle dry weight or length is expected to be influenced by intra- and interspecific weed competition because competition influences plant morphology.

For the objective of the study, optimizing the estimation of seed production, data of the number of seeds per panicle trait of *E. crus-galli* were compared on two scales, i.e. large scale (USA *vs*. Europe) and small scale (field within Germany). For seed production per panicle, the relations determined by Norris (1992b) were calibrated with experimental data from different fields in north-eastern Germany. To estimate the implications of using different relationships

for seeds per panicle, these relationships were applied to more of our field experimental data, plants per area and estimated their seed production.

3.3 Materials and Methods

Field management

Field experiments were conducted in three commercial maize fields that were minimally tilled and had been under continuous maize cultivation for at least three years. Field management was similar among all three fields. On 4-7 May 2015, maize (9 seeds m^{-2}) was sown in rows 75 cm apart and 5 cm deep in the soil. Before maize seeding, the seedbed was prepared, but only in the rows, using a rotary tiller (16 cm width). This ensured that the soil between rows remained undisturbed and that weed seeds moved neither horizontally nor vertically. Similarly, to avoid movement of seeds, no organic fertilizer was applied prior to crop sowing. The crop was fertilized with mineral fertilizer (field 1, 20 kg/ha N and 40 kg/ha P; field 2, 140 kg/ha N and 60 kg/ha K; field 3, 70 kg/ha N and 70 kg/ha P) approximately four weeks after sowing, when the maize plants had three leaves. In the middle of September 2015, the height of three randomly chosen maize plants per plot (see below) was measured, and the mean height (± SE) was calculated to be 168 ± 2 cm (n = 216) in field 1, 200 ± 1 cm (n = 216) in field 2 and 186 ± 2 cm (n = 211) in field 3. For more details about field properties, such as soil types and locations, see Pannwitt *et al.* (2017).

Experimental set-up

In 2015, seed production was measured in a completely randomized block design. Each field consisted of six blocks (10.5 × 13.5 m) with 12 plots (1.5 × 1.5 m) each that were 10 m apart. Different densities of seeds of *E. crus-galli* (300, 600, 1200, and 2400 seeds per m^2; Appels Wilde Samen GmbH, Darmstadt, Germany) were applied to two plots per block in August 2014. No seeds were added (control) in four plots per block. Seed predators, such as carabid beetles and rodents, can be active in the fields and affect the density of applied seeds. Therefore, they were excluded from half of the plots by a 60-cm-high plastic frame.

In the spring and summer of 2015, following seed addition in 2014, the effect of interspecific competition with other weed species on seed production of *E. crus-galli* was tested by 1) eliminating all other weeds (treatment 1) or 2) allowing other weeds after the crops had three leaves (treatment 2). For this purpose, half the blocks were kept weed free (except for *E. crus-galli*) by a combination of selective herbicide treatments and hand weeding (see below), while in the other half, weed management stopped after 1-4 June. Two weeks before crop

seeding, a non-selective herbicide (Glyphosate, 450 g a.i. L^{-1}, Glyphos Supreme, FMC, Germany) was applied in both treatments. This treatment most likely had no effect on *E. crus-galli* because at that time, no seedlings of *E. crus-galli* had emerged. When the crop had developed three leaves (1-4 June), leaf- and soil-active herbicides (Tritosulfuron, 250 g a.i. kg^{-1}, Arrat, BASF, Germany, and Dicamba, 500 g a.i. kg^{-1}, Dash, BASF, Germany) were applied in both treatments. When the crop had six leaves (29-30 June), a leaf-active herbicide (Bromoxynil, 225 g a.i. L^{-1}, Bromotril 225 EC, ADAMA, Germany) was applied additionally, but only to treatment 1. After that, late-emerging weeds of species other than *E. crus-galli* were manually cut to ground level every second week in treatment 1.

To test the effect of the timing of seedling emergence (cohorts), seedlings of *E. crus-galli* were marked using a differently coloured toothpick for each emergence cohort, every second week. Cohort 1 included individuals of *E. crus-galli* that had emerged before maize planting on 4 May; cohort 2 included individuals counted from 5 May until 1 June; cohort 3 included individuals that emerged between 2 and 30 June; and cohort 4 included individuals that emerged between 1 and 31 July. Seedlings that emerged in August were not considered for analysis because these plants did not produce seeds. Similarly, seedlings that emerged immediately after sowing of *E. crus-galli* in the autumn of 2014 were not considered because they died in winter and produced no seeds.

Measuring seed production

Panicles were checked for flowering from July to October 2015. Each flowering panicle was wrapped in a perforated and air-permeable bag (Crispac bag, 150 x 305 mm, pores ∅ 2.00 mm, Baumann Saatzuchtbedarf, Waldenburg, Germany) to avoid seed losses. All panicles were cut and collected a few days before maize harvest. The number of adult plants, i.e. plants that produced panicles, were counted per cohort and plot. Panicles were separated from the culm of the adult plants by cutting them approximately one cm below their lowest rachis. Panicles were oven dried (30 °C) for 24 h and stored at room temperature until they were analysed.

A total of 6491 panicles of *E. crus-galli* were harvested. For each panicle, the dry weight was determined by weighing to an accuracy of ± 10 mg and length was measured from the attachment point of the lowest rachis to the tip of the panicle.

To determine the relationship between seed number and panicle dry weight or length, a subsample of 178 panicles was drawn from all panicles. The sample was not completely random; the subsample always included small-, medium- and large-sized panicles from all treatments and all cohorts. The number of caryopses per panicle, which we refer to as seeds

per panicle, was determined by stripping the seeds from the panicles and separating apparently broken or empty seeds from intact (full, sound, and heavy) seeds and counting the intact seeds manually.

Data analysis

To select the best predictor of the number of seeds per panicle, regression models of log-transformed panicle dry weight and log-transformed panicle length were analysed separately, including their respective second-degree polynomials. Seed predators were able to access ripe, heavy panicles hanging outside the plastic frames in field 1; therefore, plots with frames from field 1 were excluded from regression analysis. Models that could accommodate skewed error distributions were tested: (1) a generalized linear regression model (GLM) with a quasi-poisson distribution; (2) a GLM with a negative binomial distribution; (3) a linear regression model (LM) after Box-Cox transformation of the response variable; and (4) a LM after log transformation of the response variable. Explanatory variables included weed cohort (1-4), field (1-3), weed seed density (300, 600, 1200, or 2400 seeds per m^2), interspecific competition with other weed species (+/-), weed seed predation (+/-) and first-order interactions. Model selection was done via backward selection using the F-test (models 1, 3, and 4) or the Chi^2 test (model 2), with $\alpha \leq 0.01$ as the test criterion.

When all data were analysed together, panicle dry weight did better than panicle length in describing seed production per panicle. Therefore, further analyses focussed on panicle dry weight as the main predicting variable of seed count. Because of significant interactions between field and panicle dry weight, further field-specific model selection and subsequent analysis were carried out. All final models met the model assumptions (linear relationship assumption, normal distribution of residuals, homoscedasticity, and absence of influential values).

Calibrating seed production data from Norris (1992b)

To compare our *E. crus-galli* seed data from Germany with data from California (Norris, 1992b), we combined and converted Norris's model equations. Norris related the number of florets and the seed dry weight per panicle to the structural dry weight of the panicle, i.e., the dry weight without the seeds, as follows:

$$\left\{ \begin{array}{c} \log(numflor) = 0.98 + 0.96 * \log(strucbiom) \\ \log(weightseeds) = 1.12 + 1.03 * \log(strucbiom) \end{array} \right\} , \quad (1)$$

where *numflor* = number of total florets per panicle, *weightseeds* = weight of seeds + aborted seeds per panicle (mg/panicle), and *strucbiom* = panicle structural dry weight (mg/panicle). From (1), it follows that the total panicle dry weight (B), i.e., the sum of the dry weight of the structure, the seeds and the aborted seeds, is

$$\mathrm{B} = exp(1.12 + 1.03 * log(strucbiom)) + strucbiom\ . \quad (2)$$

Under the assumption that *numflor* is equal to the number of seeds (*S*), it follows from (1) that

$$log(strucbiom) = (log(S) - 0.98)/0.96 \quad . \quad (3)$$

The insertion of equation (3) into equation (2), subsequent simplification and changing the units to (g/panicle) results in

$$log(\mathrm{B}) = log((exp(1.073 * log(\mathrm{S}) + 0.069) + exp(1.042 * log(\mathrm{S}) - 1.021))/1000) \quad . \quad (4)$$

Solving equation (4) for *S* is analytically impossible. Therefore, equation (4) was used to compare Norris's and our results graphically (Fig. 1).

Using the regression models to estimate seed production

To provide an example of an application of our regression models, the number of seeds produced per plant and the number of seeds produced per m^2 were estimated. This was done only for plots that excluded seed predators and had been seeded with 600 seeds per m^2 (six plots per field). We had no information on the number of panicles per individual adult *E. crus-galli* plant. However, for each plot, information was available on a) the number of *E. crus-galli* plants per cohort and per m^2, b) the proportion of these plants that were adult, c) the number of panicles per m^2 and to which cohort they belonged, d) the dry weight of each of these panicles, and e) our regression lines relating panicle dry weight to seeds per panicle based on a sample of these panicles. This allowed the estimation of means and standard errors (n = 6). The information was used as follows: the selected regression lines relating panicle dry weight to the number of seeds per panicle (Fig. 1) were used to estimate the number of seeds for each panicle (n = 896) that was bagged in any of the plots. This process was repeated using the

relationship established by Norris (1992b). Next, for each field, seed production per cohort and per m^2 were calculated by adding up the seeds produced by all panicles per field and cohort and per m^2. The number of seeds per plant is the number of seeds per m^2 divided by the number of adult plants per m^2. Then, the number of panicles per adult plant was calculated as the number of panicles per m^2 divided by the number of adult plants per m^2 per field and cohort. The number of seeds per panicle was calculated by the number of seeds per adult plant divided by the number of panicles per adult plant for each field and cohort. The calculation of the number of seeds per panicle and seeds per m^2 was repeated by using the relationship established by Norris.

Statistic tools used

All analyses were done in R version 3.1.2 (R Core Team, 2017). The package "MASS" (Venables & Ripley, 2002) was used for the negative binomial model, and the package "emmeans" (Lenth, 2018) was used to compare the slopes of different regression models.

3.4 Results

Relationship between panicle dry weight and seed production on a small scale

Of all tested models, the LM with log transformation was the most parsimonious and revealed the highest R^2 or pseudo-R^2 (explained deviance of GLMs), thus further analysis focussed on LM. When data of all fields were analysed together, the number of seeds per panicle was best described by panicle dry weight (R^2 = 92%). Using panicle length instead of dry weight as an explanatory variable explained less of the variance (R^2 = 69%) (Table 7). Analysis per field showed that only the tested variable on panicle traits, namely panicle dry weight and panicle length could explain the number of seeds per panicle. All other tested variables, i.e. weed cohort, weed seed density, interspecific competition with other weed species, weed seed predation and first-order interactions, did not significantly explain the number of seeds per panicle.

Table 7 Analysis of variance of best-selected linear regression model to describe the log number of seeds per panicle by plant traits (panicle dry weight (B) or panicle length (L)) and other explanatory variables (field (1-3), weed seed predation (+/-) and first-order interactions).

Parameter	**df**	***F* value**	***P* value**
panicle dry weight (R^2 = 92%)			
log(B)	1	1724.617	< 0.001
log(B²)	1	18.475	< 0.001
field	2	5.120	0.007
weed seed predation (+ /-)	1	0.622	0.431
log(B) x field	2	21.741	< 0.001
log(B) x weed seed predation (+ /-)	1	12.878	< 0.001
log(B²) x field	2	9.412	< 0.001
Residuals	167		
panicle length (R^2 = 69%)			
log(L)	1	309.163	< 0.001
log(L²)	1	8.910	< 0.001
field	2	0.882	0.144
log(L) x field	2	5.506	< 0.001
log(L²) x field	2	3.064	0.001
Residuals	169		

Field-specific models based on panicle dry weight are shown in Figure 4. In all fields, the number of seeds increased with panicle dry weight, but the slopes of the regression lines differed significantly among all three fields (P (χ^2) < 0.05). This indicates that plants differed between fields in the allocation of resources to seeds.

Relationship between panicle dry weight and seed production on a large scale

The confidence interval of our regression models differed significantly from the model described by Norris (1992b) in two of the three fields. If the regression model by Norris (1992b) would have been used, then seed production would have been underestimated for lighter panicles (≤ 0.3 g panicle dry weight) and overestimated for heavier panicles (≥ 0.7 g panicle dry weight) in field 1. In contrast, in field 2, seed production would have been overestimated for lighter panicles (≤ 0.5 g panicle dry weight) and underestimated for heavier panicles (between 0.9 and 3.5 g panicle dry weight).

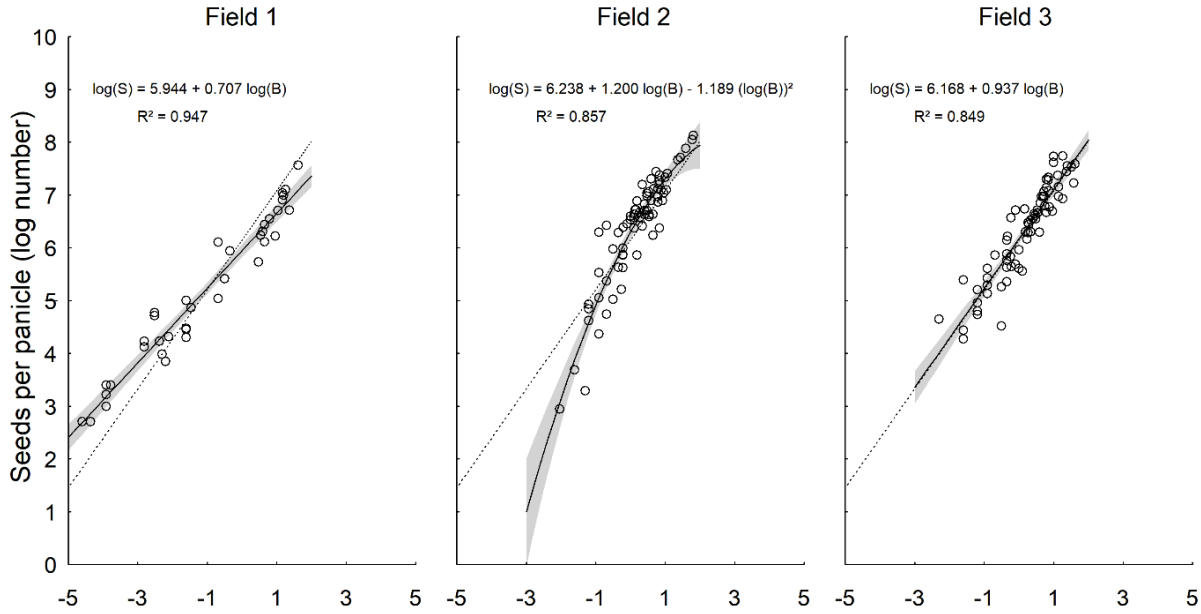

Figure 4 Number of seeds (*S*) per panicle of *E. crus-galli* depending on panicle dry weight (*B*) in three fields. Regression line with 95% confidence interval. The dotted lines display the relationship derived from Norris (1992b) ($\log(B) = \log((\exp(1.073 * \log(S) + 0.069) + \exp(1.042 * \log(S) - 1.021))/1000)$).

Using the regression models to estimate seed production

Seed production by *E. crus-galli* differed between fields as the adult plants in field 1 produced, on average, 590 (± 161) seeds per plant (n = 6); those in field 2 produced 1638 (± 403) seeds per plant; and those in field 3 produced 2483 (± 341) seeds per plant. Lowest seed production was obtained in field 1 (58157 ± 8064 seeds per m^2), followed by field 2 (130888 ± 16960 seeds per m^2), and being highest in field 3 (203643 ± 37739 seeds per m^2). The total number of adult plants per m^2, the number of panicles per adult plant and the number of seeds per panicle were higher in field 3 than in fields 1 and 2 (Table 8). In field 2, the number of panicles per adult plant and the number of seeds per panicle was higher than field 1 (Table 8).

In all three fields, the main contributor to seed production per m^2 was the number of plants that emerged in cohort 2 (field 1, 77%; field 2, 78%; and field 3, 54%), followed by the number that emerged in cohort 3 (field 1, 22%; field 2, 10%; and field 3, 46%). In field 2, seeds from plants that emerged in cohort 1 contributed only 12% to seed production, and in field 1, cohort 4 contributed only 1%. The number of panicles per adult plant and number of seeds per panicle gradually decreased, comparing all fields, in the following order: cohort 2 > cohort 3 ≈ cohort 1 > cohort 4 (Table 8).

Table 8 Adult plants per m^2, panicles per adult plant, seeds per panicle, and seeds per m^2 for each cohort (1, early May; 2, May until the beginning of June; 3, June; and 4, July) of *E. crus-galli* in three fields (1, 2, and 3) at a sowing density of 600 seeds per m^2. Seeds per panicle and the number of seeds per m^2 were estimated by regression models in each field and the regression model from Norris (Fig. 1) (n = 6; field 2, cohort 1, n = 2) (mean ± standard error).

Field	Cohort	Plants [m^{-2}]	Panicles [plant^{-1}]	Seeds [panicle^{-1}]	Seeds [m^{-2}]	Seeds in Norris [panicle^{-1}]	Seeds in Norris [m^{-2}]
1	2	31 ± 5.232	2 ± 0.132	586 ± 45	45028 ± 8732	862 ± 81	65941 ± 13232
	3	66 ± 12.426	1 ± 0.138	192 ± 47	12934 ± 2386	245 ± 67	16183 ± 3285
	4	9 ± 2.996	1 ± 0.066	22 ± 6	195 ± 92	12 ± 5	109 ± 63
2	1	16 ± 4.000	2 ± 0.000	514 ± 64	15912 ± 2054	444 ± 47	13824 ± 2050
	2	48 ± 9.179	3 ± 0.660	761 ± 78	102284 ± 16027	651 ± 63	87466 ± 13226
	3	15 ± 3.040	1 ± 0.359	517 ± 103	12692 ± 5487	450 ± 80	11054 ± 4642
3	2	22 ± 8.983	4 ± 0.877	852 ± 101	109462 ± 50090	830 ± 100	106687 ± 48815
	3	61 ± 6.746	2 ± 0.596	621 ± 46	94181 ± 18764	606 ± 45	91718 ± 18278

3.5 Discussion

The objective to optimise the estimation of seed production calls for a solid method, delivering results not affected by the plasticity of weed species due to competition or time of emergence, environmental variations and genetic differences. Moreover, it should be easy to handle. We first discuss how robust the methods based on panicle traits, namely dry mass and length, are to estimate seed production per panicle on a large scale (i.e. across continents) and then compare the differences occurring on a small scale (i.e. across fields). We then evaluate the methods to assess seed production in light of its foreseen purpose of integrated weed management.

Relationship between panicle dry weight and seed production on a large scale

Comparing Norris´ model with the model we developed, the relationship between panicle dry weight and seed production per panicle was equally closely related among the two climates, different field managements, differences in the competition with the crop and populations of *E. crus-galli* between the two continents (Europe and USA), and was not influenced by the year of the study (1992 vs. 2015). Panicle dry weight explained seed production per panicle in both models equally well. The models developed in this study, describing the number of seeds per panicle as a function of panicle dry weight or length, were not influenced by the plasticity of *E. crus-galli,* i.e. variation in plant morphology due to intra- and interspecific competition and time of emergence. Panicle dry weight, however, predicted seed production per panicle more precisely than did panicle length. Thus, on a large scale, panicle dry weight resulted in a more robust model for the estimation of seed production than panicle length.

Relationship between panicle dry weight and seed production on a small scale

However, when comparing the relationship between panicle dry weight and seed production per panicle on a small scale, the slopes of the relationship varied between the three experimental sites. When seed production was estimated in our fields using the model developed by Norris, very different estimates were obtained for two of the fields than when our own models were used. Apparently, differences on a small scale can have consequences for the total amount of seeds produced. Seed production per m^2 would have been overestimated by 41% and underestimated by 14% in fields 1 and 2, respectively, if the equation developed by Norris (1992b) would have been used.

On a small scale, competition by other weeds, seed density, or presence/absence of seed predators could not explain differences in seed production. This indicates that other factors influencing growing conditions in each field altered the slope of the relationship between seed number and panicle dry weight, resulting in field-specific values. While growing conditions appeared to alter panicle dry weight, the number of seeds per panicle always remained closely related to panicle dry weight. Different field conditions caused shifts in the timing of seedling emergence, the number of panicles per plant and the number of seeds per panicle. As expected, the timing of seedling emergence influenced seed production of the adult plants of *E. crus-galli.* With delayed emergence, seed production declined because late-emerging plants produced fewer panicles per plant and fewer seeds per panicle. Similar effects have been described for *E. crus-galli* in maize, rice and cotton fields where late-emerging weeds had to

compete with the crop, especially for light (Norris, 1992a, 1996; Bosnic & Swanton, 1997; Clay *et al.*, 2005; Bagavathiannan *et al.*, 2012). Thus, our analyses showed that growing conditions clearly altered panicle dry weight; but in each field, the number of seeds per panicle was always closely related to the panicle dry weight.

In summary, the relationship between panicle dry weight and the number of seeds per panicle appears to be surprisingly solid when compared at a large, continental scale, but can differ at a small, regional scale.

The objective to establish a reliable and straightforward method to estimate seed production is difficult to achieve in the case of *E. crus-galli*. In this study, panicle dry weight did better than panicle length in predicting seed production per panicle. The difference in the percentage of explained variance was substantial with 69% (panicle length) and 92% (panicle dry weight). In contrast to the study by Norris (1992b), in this study the total panicle dry weight was measured by including the dry weight of the seeds. This way of measuring panicle dry weight requires a much better timing and is more time consuming than simply measuring panicle dry weight without seeds or panicle length, which can be measured after seed shed. Alternatively, panicle dry weight excluding the seeds is less time consuming. Norris (1992b) waited until full seed shed, which lasted for approximately 3 weeks, to measure the number of seeds by panicle length and panicle dry weight without seeds. By using either length or dry weight without seeds as independent variables, Norris (1992b) found no difference in the explained variance (94%) of the models for seeds produced per panicle. Compared to Norris's study, the growth of *E. crus-galli* in the current experiment was limited by several variables, such as different densities of *E. crus-galli*, presence of the crop, herbicide application and seed predation. During model selection, each of these variables was dropped one at a time. Intra- and interspecific competition, however, changed the relationship between seeds per panicle and panicle length in a way that made the error in the model increase. Measuring panicle dry weight including seeds, however, requires that all seeds are still on the panicle. This method is more appropriate for *E. crus galli* plants grown under arable cropping conditions. Panicles of *E. crus-galli* were formed over a period of up to 9 weeks and the seeds did not mature simultaneously. This requires either frequent sampling or bagging the panicles, as done in this experiment. Seeds in this study did not fully shed until harvest. Rubbing the panicle to separate fixed seeds is again time consuming and can partly destroy the panicle structure.

Our study gives new insights into the pros and cons of different methods that can be used to estimate seed production in *E. crus-galli.* Both methods, i.e. based on a relationship

between seed number and panicle dry weight or panicle length, have their advantages and disadvantages, depending on the users demand for precision and work load.

(1) Panicle length can be used if the estimate of seed production does not have to be very precise or seeds have already shed. This method would be sufficient if a quick and rough estimate of seed production is required, for instance to compare the efficiency of weed control measures within a field.

(2) Total panicle dry weight is a more precise estimator of seed production. This method should be used when high accuracy and precision is needed and sufficient time and labour is available. We recommend it as the method of choice in research if different influences within one field on weed demography should be modelled, such as crop management (e.g. mechanical weed control) or seed losses caused by seed predation (Pannwitt *et al.*, 2017).

(3) Applications of population dynamic simulation models intend to predict long-term developments. Field-specific calibration of seed production is an unfulfillable request for this type of application. Even so, long-term simulation models can profit from the results of this study, as the correspondence of estimated seeds per panicle with Norris and our data are good news for these applications. Predicting seed production very precisely and accurately is valuable in itself, but cannot replace weak data on numerous other life-cycle parameters requested in these simulation models. We conclude that the accuracy in predicting seed production based on panicle traits is satisfying for their purposes. In long-term scenario applications, research resources should be allocated sensibly to quantify all population dynamic parameters.

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Chapter 4

Density-dependent processes in *Echinochloa crus-galli* compensate high seed losses by post-dispersal seed predation

Density-dependent processes in the demography in the weed *Echinochloa crus-galli* compensate high seed losses by post-dispersal seed predation

4.1 Summary

Weed management that targets the seed stage reduces the size of the ambient seedbank, but its effects on subsequent life stages are largely unknown. Post-dispersal seed predation reduces the number of seeds from the soil surface before the seeds can contribute to the seedbank. Density-dependent processes can level off the effect of seed predation in subsequent life stages. In this study, we tested if (i) targeting the seed stage affects the subsequent seedling stage, (ii) lower seedling abundance is compensated by lower density-dependent mortality in subsequent life stages, and (iii) seed predation would not affect the magnitude of final seed production, but the level would vary between fields. Therefore, we constructed a model and fully parameterised it for the summer-annual weed *Echinochloa crus-galli*, with field data from three maize fields in north-eastern Germany, in the presence or absence of seed predation and different population levels of the species. Seeds of *E. crus-galli* were applied in autumn and the number of seedlings, adult plants, and seed production per m^2 were determined the following season.
Seed predation reduced the number of seedlings. However, density-dependent mortality during the seedling stage increased fecundity with decreasing seedling density, and, thus, compensated for lower numbers of seedlings. The final level of seed production per m^2 was not determined by seed predation and initial population densities, but varied between fields. Thus, to limit *E. crus-galli* population growth, targeting the seed stage is not enough.

Keywords: *Echinochloa crus-galli*, ecosystem service, density-dependence, constant final yield

4.2 Introduction

The demography of annual plant species that reproduce by seeds can be described by their different life stages. They germinate from seeds, establish flowering adult plants, set seeds that are disseminated on the ground, and die (Cousens & Mortimer, 1995). The primary source for the reproduction of an annual plant population is the weed seedbank. Targeting the seed stage and reducing the size of the seedbank limit the establishment of plants from seeds (Davis, 2006; Gallandt, 2006). Weed management, however, mainly targets the seedling stage, since

seedlings are easy to locate and vulnerable to disturbance (Davis, 2006). Some seedlings can survive weed control, making management ineffective. The effectiveness of management depends on the potential to cause mortality in the targeted stage, and the magnitude of this mortality effect on individuals at non-target stages. In particular, density-dependent processes can compensate for lower numbers at a particular life-cycle stage. Thus, the effects of a specific management strategy on all life stages of an annual weed must be considered.

Post-dispersal seed consumption targets the seed stage, which, hence, prevents the contribution of seeds to the seedbank. Annual seed losses vary between 8 and 70% (Westerman *et al.*, 2003; Davis *et al.*, 2011), and depend on weed species (Moles *et al.*, 2003; Gaba *et al.*, 2019), seed density (Westerman *et al.*, 2008; Baraibar *et al.*, 2012; Daedlow *et al.*, 2014; Pannwitt *et al.*, 2017), type of seed predator (Hulme, 1994), field management (Trichard *et al.*, 2013) and landscape complexity (Fischer *et al.*, 2011; Petit *et al.*, 2017). Annual seed losses to seed predation can be substantial, but empirical evidence for the effect on weed population density is scarce. A modelling approach showed that weed populations decline if annual seed losses are at least 40% (Firbank & Watkinson, 1985; Westerman *et al.*, 2005). To our knowledge, experimental approaches addressing the effect of post-dispersal seed predation on other life stages are limited to the seedling stage (White *et al.*, 2007; Blubaugh & Kaplan, 2016). Experiments demonstrating effects on other non-target life stages would fill a knowledge gap (Larios *et al.*, 2017; Petit *et al.*, 2018). The understanding of the impact of seed predation on each non-target life stage would clarify its potential for weed control.

While seed predation can cause substantial seed losses, its effectiveness to manage weeds fails if density-dependent processes compensate these losses (Hulme, 1998). In density-dependent processes, mortality rates change with the population density (Holst *et al.*, 2007). Density-dependence has been recognised to influence different plant population life stages, such as seedling emergence (Watkinson *et al.*, 2000), seedling survival (Yoda *et al.*, 1963; Palmblad, 1968) and fecundity (Buckley *et al.*, 2001; Holst *et al.*, 2007). For example, the fecundity of individual plants decreases if they grow at high densities. This pattern also ensures that individual plants compensate for seed losses. The result is density-independent seed production per unit area, an effect that is referred to as the law of constant final yield (Kira *et al.*, 1953). The level of the final constant seed production, however, varies between fields because of filtering of the magnitude of seedling survival and fecundity by local abiotic environments (Wortman *et al.*, 2012).

Seed predation is likely to contribute to weed management if the weed seedbank is low and weed species regenerate by seeds (Hulme, 1998). Weed species can buffer post-dispersal

seed predation by a seedbank that is not available to seed predators. As a consequence, seed predation would be ineffective as a weed-control measure as the abundance of germinated seeds is not reduced. *Echinochloa crus-galli* (L.) P. Beauv., a typical summer-annual weed in maize fields in Germany (Redwitz & Gerowitt, 2018), propagates via seeds. The species completes its life cycle within one cropping period. The weed easily adapts to increasing competition by reducing tillers and panicles (Maun & Barrett, 1986). One single plant can produce between 2000 and 400 000 seeds (Maun & Barrett, 1986; Norris, 1992; Pannwitt *et al.*, 2019). The management of *E. crus-galli* relies mainly on chemical control targeting the seedling stage. Herbicide resistance has been found for *E. crus-galli* (Claerhout *et al.*, 2015), which calls for the use of additional alternative management strategies. Seed predation may contribute to the management of *E. crus-galli*. The response of *E. crus-galli* to seed predation, however, has not been examined to date.

In the present study, we quantified the demography of *E. crus-galli* in relation to whether or not the seed stage was targeted by post-dispersal seed predation. In north-eastern Germany, a field experiment was conducted in three intensively managed agricultural fields (continuous maize crops that were minimally tilled) with no seedbank of *E. crus-galli*. *Echinochloa crus-galli* populations were introduced to the fields at different densities, and with or without shielding from seed predation. For each population, the counts in each life stage (spring seedbank, seedling, adult, seed production per unit area and per individual plant) were determined and used to estimate the transition probability between life stages as a function of seed density. Next, the effects of density-dependent mortality on the different life stages were estimated.

In this study system, seed predation over the winter was density independent and at a high level (Pannwitt *et al.*, 2017). Hence, in the presence of seed predation, we assumed that the abundance of weeds in the next season would be reduced. We hypothesised that:

(i) Targeting the seed stage will lower the density of seedlings.

(ii) Density-dependent mortality and fecundity will compensate for the lower number of seedlings.

(iii) Seed predation will not affect the final constant level of seed production per unit area, but the level would vary between fields.

4.3 Materials and Methods

Study sites and experimental design

The experiment was carried out in three maize fields in north-eastern Germany. All three fields had a history of at least three years of continuous maize cultivation with minimal tilling. Before the start of the experiment, soil sampling indicated that *E. crus-galli* was absent from the seedbank. Fields differed in their location, size and soil type; field 1 (53°33´N, 11°08´E) was 11.8 ha in size and had sandy soil. Field 2 (53°97´N, 11°98´E) was 10.1 ha in size and its soil was loamy sand; field 3 (54°02´N, 12°02´E) was 7 ha and its soil was sandy loam. In August 2014, *E. crus-galli* seeds were added at five different densities (0, 300, 600, 1200, 2400 seeds m^{-2}; seed supplied by Appels Wilde Samen GmbH, Darmstadt, Germany). These densities reflect the size of typical ambient seedbanks of *E. crus-galli* in North-West Europe, ranging from 0 to 4050 seeds m^{-2} (Thompson *et al.*, 1997). The highest density corresponds to the maximum that can be handled experimentally, and is expected to induce density-dependent effects (Holst *et al.*, 2007). The time of seeding (August) coincided with the normal period of seed shedding, and was the time when seed predation mattered the most (Westerman *et al.*, 2009). The experiment had a randomized block design with three factors, namely seed predation, intra- and interspecific competition. The design consisted of six blocks (10.5 × 13.5 m), each 10 m apart, and with 12 plots (1.5 m × 1.5 m). Interspecific competition was initiated in three randomly chosen blocks by applying herbicides (not affecting *E. crus-galli*) once and twice (for more details see Pannwitt *et al*., 2019). The effect of seed predation was investigated by preventing seed predators' access to half of the plots by a 0.6 m high plastic frame. Each frame was buried 0.2 m deep into the soil. Plastic frames were installed permanently; however, they had to be removed for maize harvest in September 2014 and maize sowing in May 2015. Further field management was similar in all three fields, including seedbed preparation, maize sowing and application of fertiliser. The seedbed was prepared by mulching the soil. Maize was sown in rows 0.75 m apart with 9 seeds m^{-2}. To ensure that the applied *E. crus-galli* seeds did not move from the plots, the soil at the centre of each plot (0.5 × 0.5 m) was not disturbed either horizontally or vertically. For this reason, the application of manure before maize sowing was omitted. Instead, mineral fertiliser was applied to the crop at the three-leaf stage. The amount of applied fertiliser (field 1, 20 kg/ha N and 40 kg/ha P; field 2, 140 kg/ha N and 60 kg/ha K; field 3, 70 kg/ha N and 70 kg/ha P) was in line with general farming practices. For more details about field management (seedbed preparation, maize sowing, the type and amount

of herbicides, and harvest date) and preparation of *E. crus-galli* for sowing, see Pannwitt *et al.* (2017, 2019).

Determination of numbers and proportions of different life stages of E. crus-galli

Spring seedbank

In March 2015, soil samples were taken from the enclosed plots to estimate the depletion of seeds over the winter due to germination or mortality caused by physiological ageing and microbial attacks (Gardarin *et al.*, 2010). In the outer 50 cm wide perimeter of each plot, 40 randomly chosen soil samples were taken with an auger (∅ 2.54 cm) to a depth of 5 cm. The samples were stored at 4 °C until further processing to retrieve the seeds from the soil. The seeds in the soil samples were separated stepwise from organic material and larger sand particles using an Elutriator (Elutriator E48X; Disema, Bellvis, Spain; Wiles *et al.*, 1996), and apparently intact seeds were counted. Analysis showed that the mortality of seeds over the winter was not affected by the field they came from or the density of *E. crus-galli,* and, thus, the values were pooled to obtain a single mean proportion of 0.012 (Pannwitt *et al.*, 2017).

Seedlings

Emerged seedlings were counted in the inner 0.5 × 0.5 m of each plot. From May until August 2015, counting was repeated every other week, except in October and November 2014 and August and September 2015, when seedlings were counted once a month. Seedlings that emerged in autumn 2014 died during the winter of 2014–2015. To follow the fate of the seedlings throughout the season, each seedling was marked with a toothpick. In this study, weed seedlings were considered a single cohort, because the majority of seedlings that survived to become adult plants emerged within a narrow period between late May and June.

Adult plants

Adult plants, i.e. plants that survived the seedling stage and developed panicles, were counted prior to maize harvest in October 2015. In the inner 0.5 × 0.5 m of each plot, adult plants were cut down to ground level and the panicles separated from the plants.

Seed production

For each plot, seed production per square metre (m^{-2}) was estimated by using the relationship of the number of seeds per panicle dry weight (Pannwitt *et al.*, 2019). At the field scale, this relationship was stable across *E. crus-galli* density, time of seedling emergence, interspecific competition with other weeds and seed predation (Pannwitt et al., 2019). Depending on the

field, panicle dry weight explained 95% (field 1), 86% (field 2) and 85% (field 3) of the variability in seeds per *E. crus-galli* panicle. To prepare for estimation of seed production m^{-2}, seeds were collected by wrapping panicles in a perforated and air-permeable bag (Crispac bag, 150 × 30 mm, pores 2 mm diameter, Baumann Saatzuchtbedarf, Waldenburg, Germany) at the beginning of flowering, and the dry weight of the panicle, including seeds, was determined. Field-specific regressions of the number of seeds per panicle dry weight were used to estimate seed production m^{-2} based on the total number of seeds produced in the inner 0.5 × 0.5 m of each plot.

Seed predation

The main seed predators, trapped after sowing *E. crus-galli* in the three experimental fields, were granivorous rodents and carabid beetles. In particular, in field 1, 624 individuals of the granivorous carabid beetle, *Harpalus rufipes* (DeGeer, 1774) were caught in 16 traps during one week, and in fields 2 and 3, the omnivorous *Calathus fuscipes* (Goeze, 1777) was the most frequent, with 223 individuals trapped. In all three fields, the granivorous rodent species *Apodemus sylvaticus* (Linnaeus, 1758) was the most frequently trapped. During seed exposure between August 2014 and March 2015, the response of seed predators did not depend on *E. crus-galli* seed density. The seed predation rates varied between fields (mean ± sd; field 1, 0.62 ± 0.28; field 2, 0.38 ± 0.28; field 3, 0.70 ± 0.17; Pannwitt *et al.*, 2017).

Statistical analysis

We tested the effect of seed predation and density-dependent mortality on fecundity (seeds per plant) and the transition probability between different life stages: from the spring seedbank m^{-2} to seedlings m^{-2} (seedling emergence); from seedlings m^{-2} to adult plants m^{-2} (seedling survival); and from adult plants m^{-2} to seed production m^{-2} (seed production m^{-2}). The best-fitting linear regression models (LM) were selected after log-transformation of count data. Log-transformation is a standard method for count data (Begon *et al.*, 2006), with results being comparable to those of other studies. Explanatory variables were field (1, 2, and 3), seed predation (+/-), second-degree polynomial of initial density of the relevant life stage (log), interspecific competition with other weeds (+/-), and first order interactions. Model selection was via backward selection by using the *F*-test with $\alpha \leq 0.05$ as the test criterion. In preliminary analyses, life events did not respond to interspecific competition (seedling emergence, $F = 0.326$, df = 1, $P = 0.207$; seedling survival, $F = 0.391$, df = 1, $P = 0.108$; and seed production m^{-2}, $F = 0.8623$, df = 1, $P = 0.355$; fecundity, $F = 1.121$, df = 1, $P = 0.291$). Therefore, the

factor intraspecific competition with other weeds was excluded and the number of replicates for intraspecific competition increased from 3 to 6 blocks. K- value analysis (Begon *et al.*, 2006)

$$k = \frac{\log\,(\text{inital density})}{\log\,(\text{final density})},$$

was applied to quantify the magnitude of density-dependent mortality in life stages between fields, and in the absence and presence of seed predation. All analyses were carried out in R version 3.1.2 (R Core Team, 2017).

4.4 Results

For all fields, model selection indicated that seed predation and fields determined some life-cycle transitions, i.e. seedling emergence (F = 4.909, df = 2, P = 0.009) and fecundity (F = 8.150, df = 2, P < 0.001), but not seedling survival (F = 1.162, df = 3, P = 0.327) and seed production m^{-2} (F = 0.663, df = 2, P = 0.517). Similarly, the density-dependence of seedling emergence (F = 7.153, df = 2, P = 0.001), seedling survival (F = 7.388, df = 2, P < 0.001) and fecundity (F = 5.243, df = 2, P = 0.006), but not seed production m^{-2} (F = 0.1434, df = 3, P = 0.934), varied by field. Thus, further analysis of life transitions were carried out per field.

Seedling emergence

When analysed per field, the abundance of seedlings was reduced considerably by seed predation, which ranged from 40 to 50% in field 1, from 26 to 48% in field 2, and from 53 to 69% in field 3 (Figure 5). In the presence or absence of seed predation, seedling emergence decreased with increasing seeding density (slope [initial density] < 1;Table 9), except in field 3, where, in the absence of seed predation, seedling emergence increased with increasing seeding density (slope [initial density] > 1;Table 9). Seed mortality was higher in the presence than in the absence of seed predation. Furthermore, seed mortality increased with density (Figure 6).

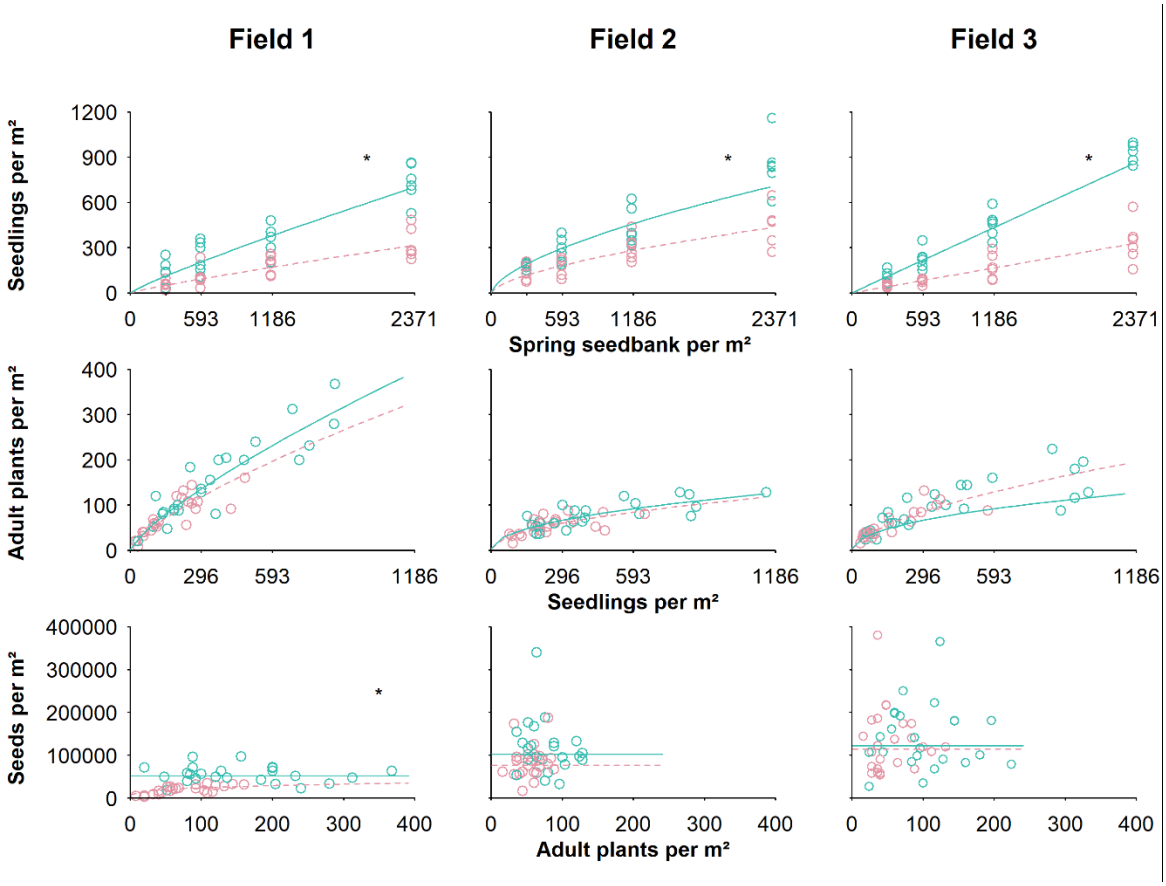

Figure 5 Back-transformed linear regression models showing the responses of transitions between life stages of *Echinochloa crus-galli*, i.e. seedling emergence, seedling survival, seed production m^{-2}, to seed predation (red circles and dashed line) over the winter vs. no seed predation (green circles and solid line) over the winter, as a function of *E. crus-galli* density, in three maize fields in north-eastern Germany (n = 6). Panels labelled (*) indicate a significantly different ($P < 0.05$) response to seed predation.

Seedling survival

In all three fields, the abundance of adult plants did not change as a result of seed predation (Figure 5). Negative density-dependent processes regulated the transition from seedlings m^{-2} to adult plants m^{-2} (Table 9). Seedling mortality was higher in the absence than in the presence of seed predation (Figure 6).

Table 9 Log-transformed parameter estimates, standard errors (SE) and R^2 values for linear regression models for *Echinochloa crus-galli* seedling emergence (r); seedling survival (s), seed production m^{-2} (sp) and seeds $plant^{-1}$ (f**)** as a function of seed predation (**pred**) and density of the relevant life-cycle stage in fields 1, 2 and 3.

Model	**Parameter**	**Field 1**			**Field 2**			**Field 3**		
		Estimate	**SE**	R^2	**Estimate**	**SE**	R^2	**Estimate**	**SE**	R^2
$\mathbf{r_{pred}}$	Intercept	-1.489	0.848	0.718*	1.755	0.633	0.605*	-1.258	0.690	0.777*
	Slope (initial density)	0.937	0.125		0.543	0.093		0.892	0.101	
r	Intercept	0.003	0.836	0.675	1.068	0.415	0.861	-1.705	0.473	0.918
	Slope (initial density)	0.834	0.123		0.718	0.061		1.102	0.069	
$\mathbf{s_{pred}}$	Intercept	0.568	0.384	0.800	1.225	0.582	0.499	1.014	0.281	0.827
	Slope (initial density)	0.736	0.078		0.502	0.107		0.601	0.058	
s	Intercept	0.521	0.582	0.834	1.495	0.504	0.583	1.192	0.412	0.746
	Slope (initial density)	0.769	0.107		0.472	0.085		0.572	0.071	
$\mathbf{sp_{pred}}$	Intercept	6.759	0.449	0.674	11.250	0.099		11.648	0.104	
	Slope (initial density)	0.726	0.107							
sp	Intercept	10.851	0.079		11.530	0.104		11.712	0.121	
$\mathbf{f_{pred}}$	Intercept	6.759	0.449	0.227*	10.528	1.037	0.307	11.371	0.804	0.481
	Slope (initial density)	-0.274	0.107		-0.817	0.262		-0.928	0.206	
f	Intercept	11.051	0.600	0.766	12.427	1.226	0.454	10.983	0.936	0.425
	Slope (initial density)	-1.041	0.122		-1.220	0.285		-0.837	0.208	

pred = with seed predation; an R^2-value with an asterisk (*) indicates that seed predation differed significantly within the relevant life stage ($P < 0.05$)

Fecundity

In field 1, fewer seeds were produced per plant in the presence of seed predation. However, fecundity did not differ between fields 2 and 3. In all three fields, fecundity was negatively density-dependent (Table 9). The level of plant mortality was higher in field 1 than in fields 2 and 3. Density-dependent plant mortality was higher in the absence than in the presence of seed predation in field 3 (Figure 6).

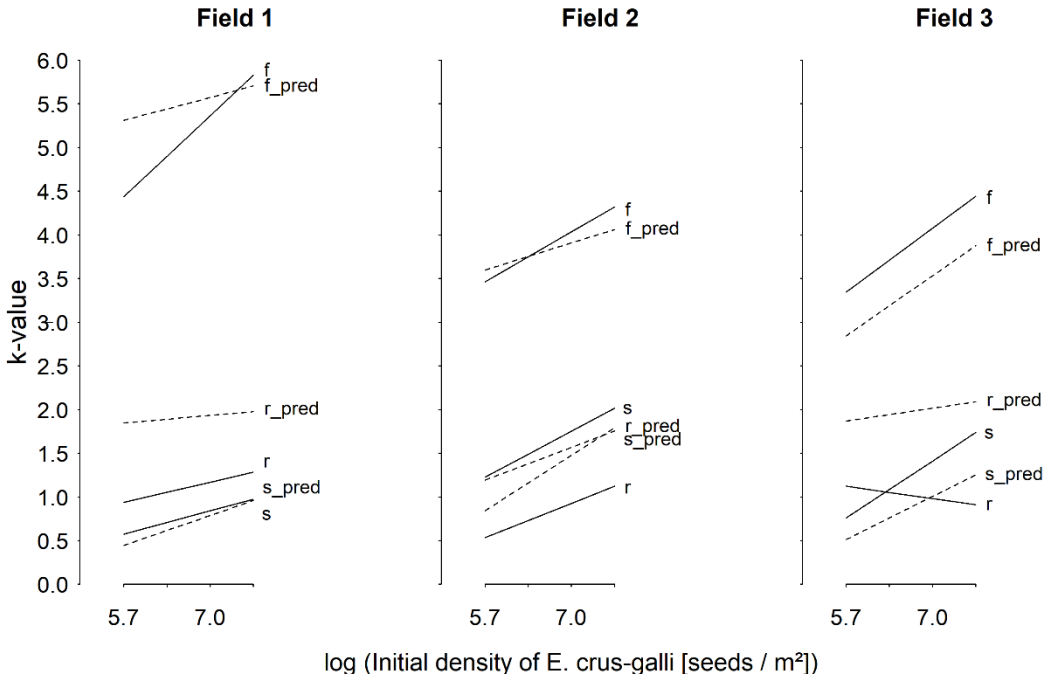

Figure 6 K-values for seedling emergence (r), seedling survival (s), and seed production per plant (f) of *Echinochloa crus-galli* in the presence ("pred", dashed lines) or absence (solid lines) of seed predation in fields 1, 2, and 3.

Seed production

Seed production m^{-2} was influenced neither by seed predation nor by the density of adult plants, except in field 1 where seed production m^{-2} was lower in the presence than in the absence of seed predation, and negatively density-dependent (Figure 5;Table 9)The estimated seed production m^{-2} varied between the fields, i.e. lowest in field 1 (51 621 seeds m^{-2}), intermediate in field 2 (101 744 seeds m^{-2}) and highest in field 3 (122 098 seeds m^{-2}).

4.5 Discussion

Seed mortality influences numbers in the subsequent seedling stage

As we had expected in our first hypothesis, mortality at the seed stage influenced counts at the subsequent seedling stage. In addition, the high mortality of seeds due to seed predation (≤ 77% seeds removed from experimental plots; Pannwitt *et al.*, 2017) limited seedling emergence to a similar level (26 – 69%). This pattern does not reflect the findings by Blubaugh and Kaplan (2016). In the presence of seed predation, they followed the fate of applied *Chenopodium album* L. seeds to the seedling stage. Here, seed losses limited seedling emergence by 38% only. Both studies differed in the size of the ambient seedbank. We measured the effect of seed losses on the seedling stage exclusively with freshly applied seeds, as our experimental fields did not have *E. crus-galli* seedbanks. Blubaugh and Kaplan (2016), in contrast, conducted their

experiment in a field that carried an ambient seedbank of 15 000 seeds m^{-2}. Here, seedlings emerged from both newly shed seeds and from older seeds in the ambient seedbank. Thus, mortality at the seed stage limits the number of individuals in the subsequent seedling stage, but efficiency depends on the size of the ambient seedbank.

E. crus-galli compensates for lower abundance of seedlings in subsequent life stages.

As we had expected, mortality at the seed stage limited the abundance of seedlings (≤ 69%), but this effect levelled off in subsequent life stages, namely adult plants, seed production per unit area (except in field 1) and fecundity. These results are similar to those from a study demonstrating the life cycle of an invasive plant species in the presence of pre-dispersal seed predation that targeted seeds on the plant before seed-shed (Garren & Strauss, 2009). Garren and Strauss (2009) showed that seed losses were compensated in the subsequent vegetative period.

The summer-annual weed species *E. crus-galli* compensates seed losses through density-dependence in seedling emergence, seedling survival and fecundity. Density-dependent regulation in *E. crus-galli* is in line with that in other annual weed species, i.e. *Veronica hederifolia* L., *Papaver rhoeas* L., *Fumaria officinalis* L. and *Capsella bursa-pastoris* (L.) Medik. (García de León *et al.*, 2014). In our study, plants compensated seed losses by adjusting the level of density-dependent mortality in subsequent life-cycle transitions. Thus, in *E. crus-galli* populations with a high density of seedlings (in the absence of seed predation), subsequent life-cycle transitions responded with higher density-dependent mortality than did populations with a lower density of seedlings (in the presence of seed predation). Finally, seed production m^{-2} was constant in all populations of *E. crus-galli* (but see explanation for field 1 below). The results of this study provide empirical evidence for the law of constant final yield (Kira *et al.*, 1953).

The level of the final constant seed production depends on in-field environments

The level of the final constant seed production of *E. crus-galli* varied between fields. Seed production increased from field 1 to field 3. In each field, the *E. crus-galli* population adapted to the magnitude of seedling mortality and the per capita seed number. In field 1, seedling mortality was low, while individual plants set a comparably low number of seeds. In fields 2 and 3, in contrast, both seedling mortality and fecundity were higher than in field 1. Our results are in line with other plant species, such as *Ambrosia trifida* L., in which in-field environments strongly influenced life-cycle transitions, i.e. seedling mortality and loss of fecundity

(Wortman *et al.*, 2012). The in-field environment may explain varying seed production. In field 1, soils were drier than in fields 2 and 3. Furthermore, the total plant biomass of our populations was lower in field 1 than in fields 2 and 3 (Selig *et al.*, 2018). Dry soils hamper individual plant growth, biomass (Wiese & Vandiver, 1970) and related fecundity (Thompson *et al.*, 1991; Weiner *et al.*, 2009), and, thus, the constant final seed production per unit area.

In field 1, mortality at the seed stage due to seed predation significantly influenced the final seed production. In particular, the presence of seed predation had a density-dependent effect on seed production. This result is surprising, as subsequent life stages influence each other. The previous non-target stage (adult plant stage), however, was not affected by seed predation. In the presence of seed predation, seed production was especially low when there were fewer than 40 adult plants m^{-2}. When we excluded these data from our regression models ($\leq$ 40 adult plants m^{-2}), seed production levelled off to a constant final yield. Thus, at lower adult plant density, *E. crus-galli* populations still had the capacity to grow, whereas at adult plant densities of > 40 plants, *E. crus-galli* populations could not grow further. In field 1, fecundity was lower in the presence than in the absence of seed predation. We discuss three possible explanations for this effect. First, fecundity is known to be density-dependent (Buckley *et al.*, 2001; Holst *et al.*, 2007). This implies that, in the presence of seed predation, *E. crus-galli* plants should have accumulated to a higher extent. We, however, did not observe a higher accumulation of plants in plots with seed predation compared to plots without. Second, fecundity decreases with seedling-emergence time (Clay *et al.*, 2005; Bagavathiannan *et al.*, 2012). If the differences in seed production are related to the age structure of *E. crus-galli*, we would expect a higher number of late-emerging plants in the plot with seed predation. The age structure, however, was similar in both treatments (data not shown). Third, fecundity is related to abiotic conditions, such as precipitation and temperature (Wortman *et al.*, 2012). The plastic frames we used to prevent seed predation might have caused a favourable microclimate, e.g. higher temperature and humidity. In field 1, dry conditions might have accelerated humidity within the plastic frames, resulting in higher fecundity. Thus, in-field conditions, but not management targeting the seed stage, influence final seed production, supporting our third hypothesis.

Implications for weed management

Evaluating our results from an agricultural perspective, the main issue for integrated weed management is to maintain the abundance of seedlings under a certain threshold. Thus, weeds do not cause crop yield losses that would warrant the use of additional control measures. For

E. crus-galli in maize fields, the threshold level is 6 seedlings (Lfl Bayern, 2019). In our study, seed predation clearly affected the number of seedlings, but failed to keep the weeds below this threshold. To limit the growth of a plant population, several studies suggested 80 to 100% reduction in transitions between life stages (Buckley *et al.*, 2001; Westerman *et al.*, 2005; Ramula & Buckley, 2010). For controlling weeds, simply relying on targeting the seed stage by seed predation is not enough. Model analysis testing the effect of multiple weed management strategies to reduce the reliance on herbicides in *Abutilon theophrasti* Medik. populations indicated that seed predation of 40%, combined with crop rotation, reduced herbicide use, and no-till reduced the population (Westerman *et al.*, 2005). As a consequence, efficient weed control does not rely only on the control of the seed stage, but should also target other life stages. Thus, to reduce yield losses, additional management strategies are useful. In maize crops, farmers use herbicides to control weeds at the seedling stage. In modern and integrated weed management, however, one of the principles is to use as little herbicide as possible (Barzman *et al.*, 2015). Seed predation contributes by limiting population growth and reducing seed-bank influence, by making herbicides more efficient, as herbicides target weed plants at low densities better than at high densities (Taylor & Hartzler, 2000). Thus, mortality at the seed stage can indirectly reduce the use of herbicides.

Furthermore, our results illustrate the consequences of the failure of management strategies targeting the seedling stage. To identify the minimum number of seedlings required to level off to a constant final yield, we used regression models and subsequently added seedling data until the yield was constant. We assume that if ≥ 32 *E. crus-galli* seedlings m^{-2} escape weed management in maize crops, populations can compensate these losses. The reasons for weeds escaping management strategies are diverse, but herbicide resistance is one of the most intractable problems. Ignoring seedling escapes would increase the weed population towards a constant final yield. Thus, our results give new insights that help farmers understand the behaviour of *E. crus-galli* if management fails.

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Chapter 5

General Discussion

General Discussion

In the following chapter, the results of Chapters 2 to 4 are discussed in a broader context. First, the results of Chapter 2, on the seed predation assemblage and the level and response to different weed seed densities of *Echinochloa crus-galli*, are addressed, followed by the identification of potential drivers that enhance seed predation rates in *E. crus-galli.* Next, the results of Chapters 3 and 4, on the demography of *E. crus-galli* both in the absence and presence of seed predators, are evaluated. Finally, the findings of this study are combined in order to identify integrated solutions for managing population densities of *E. crus-galli* in maize.

Seed predation community and effect on weed seed mortality

One obstacle that was adressed in this study was to understand seed predators behaviour. We identified the seed predator assemblage in maize fields, specifically the level and response of seed predators to different weed seed densities in two consecutive years. In addition, the level and response of the seed predators were tested at two different seed exposure times, namely, in the short term (August until maize harvest in September–October) and the long term (August 2014 until March 2015). We identified carabid beetles and rodents as the main seed predators in maize. In the first year, the predator assemblage was dominated by granivorous carabid beetles, and in the second, by rodents. The level of seed predation was lower when carabid beetles dominated the predator assemblage and higher when mainly rodents were present in the maize fields. The response to weed seed density was mainly inversely density dependent when carabid beetles were most abundant, which is similar to the results from previous studies conducted in wheat fields in north-eastern Germany (Baraibar *et al.*, 2012) and maize fields in the United States (Westerman *et al.*, 2008). In the second study year, almost all seeds were removed. Thus, we could not determine the response to weed seed density. In Spain, high seed predation rates have also been also reported in cereal fields when rodents were most abundant (Daedlow *et al.*, 2014). With a prolonged period of available seeds on the soil surface, the response to weed seed density changed from inversely density dependent and direct density dependent to density independent. Thus, seed predators removed seeds, being active when the fields were idle between crop harvests in autumn and the following spring.

Rodents, landscape matrix and seed exposure time can be potential drivers in increasing seed predation rates in E. crus-galli

In an agroecosystem, post-dispersal seed predation would be effective in controlling weed species if seed removal was at a high level or the response was in a directly density-dependent manner. In our study system, the seed predation rates were 11 – 77% in the first study year and 49 – 100% in the second. Davis *et al.* (2011) predicted seed predation rates to vary between 30 and 99%, and so the level of seed predation in our study system was comparatively high. Furthermore, the response of the seed predators to different weed population densities was mainly density independent. Thus, seed predation was effective, in terms of the level of seed removal, but not in terms of the response to weed seed density.

Seeking to identify what caused such a high level of seed predation in our experimental fields, we turned our attention to potential external drivers. Similarly to the study by Menalled *et al.* (2000), we found that the level of seed predation was related to the landscape matrix, increasing from simple to complex structures (Menalled *et al.*, 2000; Pannwitt *et al.*, 2019). Seed predators prefer complex habitats, as these habitats provide structures that act as refugia, protecting seed predators from being the object of predation themselves by higher taxa. Seed predators prefer these habitats while crop fields lie idle after harvest, or early in the season when the crop provides little canopy cover (Holland *et al.*, 2017; Tschumi *et al.*, 2018). Thus, complex habitat structures can be a possible driver for seed predation. The habitat structure protects seed predators from being the object of predation, and from these habitat structures, seed predators can re-enter the field with canopy cover and remove weed seed.

Another potential driver for seed consumption was the period in which the weed seeds were accessible to seed predation, with a long period of seed accessibility increasing seed removal rates. Seeds are accessible to seed predators until they are either incorporated into the soil via tillage regimes or by natural means, such as rain splash (Westerman *et al.*, 2009). In Chapter 2, we compared the effect of two different seed exposure times on the level and response of the seed predators. The seed exposure times differed in length (short and long term), and the presence of canopy cover by the maize crop. The results showed that the level of seed predation slightly increased and the response to different seed densities of *E. crus-galli* changed from the short- to long-term seed exposure times. In particular, the response changed from inversely or directly density dependent to density independent. The seeds might have been accessible to the seed predators during the short-term exposure because the canopy cover of maize protected the weed seeds from rain splash and, consequently, incorporation into the

soil. In the long term, the seeds might have been accessible to the seed predators until they were incorporated into the soil via tillage the next spring. Thus, the level and response of seed predation to different weed seed densities can increase or change, respectively, when soil movement by tillage and crop harvesting is delayed.

While the seed predation rate was relatively high in our study system, it did vary between years. In the year with a high level of seed predation, rodents dominated the predator assemblage. A high level of seed removal and a direct density-dependent response to density populations is known to be associated with a dominance of rodents in the seed predator assemblage (Westerman *et al.*, 2008; Daedlow *et al.*, 2014; Tschumi *et al.*, 2018). The results presented in Chapter 2 confirm that rodents are an important driver for effective weed seed predation. Unfortunately, the abundance of rodents fluctuates between years and, to date, data clearly indicating the factors that cause fluctuations in granivorous rodent abundance in agroecosystems is missing. Such information would help in the evaluation and promotion of the weed control potential of rodents.

Density-dependent processes regulate the demography of E. crus-galli

This section concentrates on the flexible interaction of demographic stages in different population densities of *E. crus-galli*, but it does not touch upon the influence of seed predators. The results showed that the different population densities of *E. crus-galli* were regulated by intraspecific competition. In particular, density-dependent regulation processes limited seedling germination, seedling survival and fecundity, and yielded a constant seed production per unit area (for further details on estimating seed production in *E. crus-galli*, see Box 1). Hence, the final seed production per unit area in low population densities equals that in high

population densities. In a natural ecosystem, self-regulation in plant populations that result in a constant final yield is a well-known phenomenon (Kira *et al.*, 1953; Yoda *et al.*, 1963). In an agricultural ecosystem. where resources such as fertiliser are optimally managed in order to maximise crop yields, self-regulation in weed populations is rather surprising. In agroecosystems, individual weed plants typically grow in association with each other, in competition with the crop. Thus, one would expect intraspecific competition to be less important in agroecosystems, as individual weed plants benefit from applied resources and do not compete over them. However, the growth in density populations of *E. crus-galli* in agroecosystems are regulated by intraspecific competition, in terms of density dependence. Density-dependent regulation has also been shown in the summer-annual dicotyl weed species *Abutilon theophrasti* Medik. in soybean (Lindquist *et al.*, 1995).

Box 1: Optimising the estimation of seed production

To optimise the estimation of seed production in the highly plastic weed species *E. crus-galli*, we tested the relationship between the number of seeds per panicle and the panicle traits (panicle dry weight and panicle length) under conditions that caused plasticity in the plant morphology, as detailed in Chapter 3. We avoided seed shedding, all panicles being enclosed in a permeable plastic bag. Panicle dry weight predicted seed production per panicle most accurately and precisely, and was not affected by the plasticity in *E. crus-galli* caused by competition or time of emergence, environmental variations or genetic differences. Hence, we used the relationship between seeds per panicle and panicle dry weight to predict the seed production per unit area in *E. crus-galli*. Validation of the relationship between the number of seeds per panicle and panicle dry weight using a previous study (Norris, 1992) showed that panicle dry weight is a reliable method. Other direct methods for estimating seed production, such as sticky boards or pans, can underestimate seed production due to seed loss (Norris, 2007). Using the protocol that was validated in Chapter 3, seed losses were prevented. Thus, using this our protocol is appropriate and simplifies the estimation of seed production in *E. crus-galli*.

Echinochloa crus-galli is a highly relevant weed species in maize (De Mol *et al.*, 2015; Pannwitt *et al.*, 2018), as it belongs to the weed assemblage in dense maize cropping patterns (Redwitz & Gerowitt, 2018) and causes high yield losses (Bosnic & Swanton, 1997). This summer-annual weed species has demonstrated that it is flexible enough to cope with competition and various environmental conditions in tested maize fields. A key factor that ensures the persistence of *E. crus-galli* in the seedbank is its ability to germinate over the whole vegetative period of maize. Independent of the time of germination, its plants are able to

reproduce. Early-emerging cohorts, however, have the greatest impact on the seed production of the population densities, and cause high yield losses. Late-emerging cohorts of *E. crus-galli* have the lowest impact on the total seed production in maize. These results are in line with results from previous studies (Bosnic & Swanton, 1997; Clay *et al.*, 2005). Seed production differs between the early- and late-emerging seedlings, as seed production is related to plant biomass (Weiner *et al.*, 2009), and early-emerging seedlings have a higher biomass than late-emerging seedlings (Selig *et al.*, 2018). The flexibility of *E. crus-galli* results in high seed production at the population level, a high population growth rate, and the spread of *E. crus-galli*. Given that maize cultivation has increased over the last several decades in Germany, and with the acreage of maize cultivation thus having been expanded, the weed *E. crus-galli* will likely become even more relevant in agroecosystems. Weed management aimed at reducing the weed population growth rate should target the seedbank (Davis, 2006). Limiting input to the seedbank could be one approach to reducing the population size of *E. crus-galli*. This approach would be effective as a weed control method if the demographic stages outlined below can be limited as well.

Density-dependent processes regulate seed losses in the demography of E. crus-galli

In Chapter 4, we evaluated the effect of limited seed input/mortality via seed predation on the demography of different population densities of *E. crus-galli*. The conservational biological control measure clearly limited the seed stage of population densities in *E. crus-galli*. Seed losses affected the subsequent seedling stage, with population densities declining from the seed to the seedling stage. The number of seedlings was reduced because seed predation reduced the seed intake into the seedbank, and only a proportion of the seedlings germinated from this reduced seedbank. In subsequent life stages, density-dependent processes regulated the lower number of seedlings. This is similar to the results that showed that seed predators did not touch the seed stage in density populations in *E. crus-galli*. Seedling survival was affected by intraspecific competition; thus, the number of adult plants was limited. The density of the adult plants regulated the fecundity, such that a low number of adult plants were compensated for by a high fecundity, and vice versa. Hence, low initial population densities had a greater capacity to grow than higher initial population densities. Compensation in the different population densities yielded a constant final seed production per unit area. Initial population densities of *E. crus-galli* multiplied the densities within one generation. Thus, seed predators secured their food sources, but failed to control the growth population densities as a single measure. These findings have been claimed to fill a knowledge gap (Menalled *et al.*,

2000; Petit *et al.*, 2018) and to increase our understanding of the weed control potential of seed predation.

Quantitative data on density-dependent demographic processes in *E. crus-galli* can help in developing population-dynamics simulation models. These models can be used to quantify the consequences of management measures for population growth in the long term (Holst *et al.*, 2007). In this case, a simulation model analysed the effect of seed predation presence or absence on the growth rate of seedbank population densities in *E. crus-galli* over multiple years. Decelerated growth in the presence of seed predation would be expected, as the input of new seeds into the seedbank would be limited each year.

Density-dependent seed predation will be mediated by density-dependent processes in E. crus-galli

Manipulating the density of weed seeds by seed predation does not influence the number of adult plants or seed production per unit area. At these life stages, population densities reached an equilibrium, with the level of density-dependent mortality in the seedlings and the fecundity being higher in the absence, rather than in the presence, of seed predation. While seed loss by seed predation was high and density independent, as shown in Chapter 2, a certain number of seeds could still contribute to the seedbank. Some of these seeds could then germinate from the seedbank in the next spring. These seedlings would be able to compensate and produce a constant final seed production in a field. Density-dependent regulation in population densities of *E. crus-galli* question some of the assumptions stated in Chapter 2 and in other studies (Westerman *et al.*, 2008; Baraibar *et al.*, 2012; Daedlow *et al.*, 2014). Here, it was assumed that seed predators would have the greatest impact on weed population densities if they removed seeds in a directly density-dependent manner. However, density-dependent regulation in the demography of *E. crus-galli* has shown that density-dependent seed predation is irrelevant in the control of *E. crus-galli*.

Managing E. crus-galli populations – identifying an integrated solution

The above-described density-dependent regulation of population densities of *E. crus-galli* is rather good news for weed managers. If seeds or seedlings of *E. crus-galli* survive weed control, they are able to compensate for their limited population densities. The weed control of *E. crus-galli* in maize mainly relies on the mortality of the seedlings using chemical methods. Factors such as the evolution of herbicide resistance in *E. crus-galli* (Claerhout *et al.*, 2015) or inadequate herbicide application can limit the effectiveness of such chemicals, favouring

seedling survival and reproduction. These facts challenge weed managers to rethink the strategies they use. One such strategy is the multi-tactics approach, which is applied during particular weed life stages to prevent weed population growth. It uses numerous control measures, including herbicides or cultural methods, to reduce the input of new seeds into the seedbank. Weed management must thus change from simply minimising yield losses in the current season to implementing preventative measures that would limit new seed input into the seedbank, thereby reducing the growth of the weed population for seasons to come (Liebman & Gallandt, 1997). In Chapter 4, it was indicated that weed managers need to address the mortality of the seedlings or reduce the fecundity in order to limit growth in the *E. crus galli* seedbank. The higher the level of mortality, the lower the final seed production per unit area. Several studies have suggested the need for an 80 to 100% reduction in the transition between life stages (Buckley *et al.*, 2001; Westerman *et al.*, 2005; Beckie, 2006; Ramula & Buckley, 2010) in order to limit the growth of a plant population. Apart from using crop rotation, which is, without question, an effective measure for reducing the seedbank (Bohan *et al.*, 2011), a multi-tactic approach, targeting different life stages, could also reduce weed population growth rates. Thus, this discussion turns next to the management of the different life stages of *E. crus-galli* in maize crops.

Numerous control measures, including herbicides and mechanical or cultural weed methods, can be used to target the seedling stage in order to limit the growth of population densities. Targeting this stage has many advantages, as seedlings are easy to detect and vulnerable to control measures, and can cause yield loss if they remain uncontrolled. Undersown cover crops is one cultural control measure used to limit the growth of seedlings (Teasdale, 1996), and to reduce the development of herbicide-resistant weeds by suppressing late-emerging seedlings (Beckie, 2006; Norsworthy *et al.*, 2012). However, in a simulation exercise, Redwitz *et al.* (2016) predicted that the suppression of late-emerging weeds by undersown cover crops could accelerate the evolution of herbicide resistance in *E. crus-galli* in maize. It was found that late-emerging weeds potentially did not undergo selection for herbicide resistance, while, in contrast, early-emerging weeds did. The suppression of late-emerging weeds thus successively reduces the number of sensitive seeds in the seedbank and accelerates the growth of a herbicide-resistant seedbank. Nonetheless, the results of this study also showed that undersown cover crops can suppress density in *E. crus-galli* populations.

Fertiliser management is another possible tactic for reducing fecundity in *E. crus-galli*. The fecundity varied between the experimental fields because in-field conditions differed in the amount of fertiliser applied. In the field with the lowest amount of applied fertiliser,

fecundity was low, and this resulted in a low level of final seed production per unit area. An affinity for nutrient-rich soils has been reported for *E. crus-galli*, which is a C4 grass, by Maun and Barrett (1986). Thus, regulating the crop/weed interaction using soil fertility management could be effective in managing weeds (Di Tomaso, 1995; Kirkland & Beckie, 1998; Blackshaw, 2005). The placement of the fertiliser can also affect weed population dynamics and competition between the crop and weeds (Liebman & Mohler, 2001; Blackshaw, 2005). Therefore, the input of fertilisers into the soil should be done in a manner that both favours the maize crop and reduces the extraction of nutrients by the weed (Kaur *et al.*, 2018). Placing the fertiliser near the crop rows instead of broadcasting it onto the soil surface has resulted in increased crop yields and reduced weed biomass in field experiments (Di Tomaso, 1995; Rasmussen *et al.*, 1996).

Where suppression of the weeds in previous life stages failed, and *E. crus-galli* was able to produce seeds, conservational biological control via seed predation limited the input of the newly-shed seeds to the seedbank. The results presented herein have indicated that seed predation, as a single measure, failed to control population densities in *E. crus-galli* because the seed losses were compensated for in a subsequent life stage. Seed predation, however, can be valuable when weed managers adopt it as one of a number of tactics. Given that *E. crus-galli* sheds seed as soon as it ripens, before the maize harvest, seed predators are able to remove the seeds as soon as they are on the soil surface. Thus, it is an effective tactic for controlling weed seeds prior to the maize harves. Alternative and effective control tactics that mimic seed predators prior to maize harvesting (Liebman *et al.*, 2016) and contribute to long-term weed management (Taylor & Hartzler, 2000; Davis, 2006) may be available. Farm technologies, such as the seed destructor, a non-chemical weed control tool that destroys 95% of the weed seeds on the mother plant during cereal harvest, has recently gained attention as a way to manage herbicide-resistant weeds in Australia (Walsh *et al.*, 2012). In maize, however, such technical devices have not yet been developed. Thus, seed predators remain one of the main tactics for limiting the input of *E. crus-galli* seeds into the seedbank in maize fields. The reduction of the seedbank consequently reduces the number of seedlings that will germinate the next spring. This lower number of seedlings, in turn, reduces the management intensity required to control the successful seedlings (Dieleman *et al.*, 1999; Taylor & Hartzler, 2000), consequently reducing the overall costs of weed management. Scientific evaluation of the economic value of seed predators is limited, but the first economic analysis, by Zhang *et al.* (in press), showed that seed predation can lower the costs associated with using herbicides.

To summarize, seed predation can be integrated into a combined approach towards controlling *E. crus-galli*. However, although seed predation rates tended to be high in our empirical study, the impact on population growth was low. To effectively control the economically important weed species *E. crus-galli* in maize, seed removal via predation needs to be combined with multiple tactics that target the seedling stage and the fecundity. Because the typical maize weed *E. crus-galli* can compensate for the loss of seeds and seedlings, it is necessary to identify and deploy effective control measures targeting the life stages.

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Thesen

On the demography of *Echinochloa crus-galli* and the effect of post-dispersal seed predation in maize fields

submitted by Heike Pannwitt

Rationales and Objectives

- Integrated weed management aims to control weed populations while reducing the use of herbicides
- This approach uses a multiple array of tools including biological, chemical, physical and cultural measures
- There are novel and promising control measures, such as post-dispersal seed predation as a conservational biological control
- *Echinochloa crus-galli* (L.) P. Beauv is one the most important weed species in maize in Germany
- The goal of this study was to identify the demography of the weed species *E. crus-galli* and the effect of post-dispersal seed predation in maize fields to test the weed control potential of seed predators
- Three main obstacles were addressed to reach the goal: seed predators behaviour, weed species *E. crus-galli* demography and seed predators effect on the demography of the weed

Methods

- Two field experiments were conducted in the pure stand of three minimally tilled and a history of 3 years consecutive maize in north-eastern Germany
- A short-term experiment was conducted to test the behaviour of seed predators and identify their episodic response to different weed seed densities of *E. crus-galli* in two subsequent years
- A long-term experiment was installed to follow the demography of *E. crus-galli* in the presence and absence of seed predation within one year

Thesen

1. Zooming into the pure stand of a maize field, this crop is habitat of beneficial biodiversity, such as different species of carabid beetles and granivorous rodents. Given that seed predators consumes weed seed on the soil surface, they limit the input of new seeds to the weed seedbank. Their assemblage differed between fields and year. If granivorous rodents were present, the effect on the weed seedbank is larger, than if carabid beetles dominated the predator assemblage.
2. In maize fields in north-eastern Germany, seed predators can reduce population densities of *E. crus-galli*. The efficiency is highest, if predation either respond in a direct density-dependent manner, meaning that seeds in a high density population have a higher probability to be consumed than in low density populations or consume seeds at a high level in all population densities. Given that the level of predation were at a relatively high level, but seed predation respond in a density - independent manner their efficiency to reduce population densities in maize fields can still be boosted to a direct density depend response.
3. Panicle traits of the highly plastic summer-annual weed species, *E. crus-galli* are appropriate to measure seed production. Both, panicle dry weight and panicle length are related to the number of seed per panicle. These relationships are not even effected by the plasticity of the weed due to competition, time of seedling emergence or genetic differences, but varied between maize fields in north-eastern Germany. As *E. crus-galli* adapt easily to weed control tactics, using panicle traits to estimate seed production supports the development of new integrated weed measures.
4. In the absence of seed predation, population densities of *E. crus-galli* depend on the density-dependent regulation in maize fields. Density-dependent mortality regulate the seedling emergence, seedling survival and per capita fecundity of *E crus-galli* and result in a constant level of seed production among the initial population densities. Thus, for an efficient control of *E. crus-galli* in maize fields, control measured should limit seedling survival and per capita fecundity.
5. Touched by seed predators, density-dependent processes compensated seed losses in population densities of *E. crus-galli*. Seed predators reduced the seedbank and number of seedlings. Density-dependent regulation, however, act on seedling survival and per capita fecundity. The number of adult plants and seed production per unit area were similar to the population densities of *E. crus-galli* that was not touched by seed predation. Thus, the effect of seed predation was leveled off. Seed predation failed as a

single measure to control the growth of population densities in *E. crus-galli*. However, seed predation limit the input of new seeds into the seedbank, the dissemination of the seeds, reduced the number seedlings and thus enhance the effectiveness of control measured targeting the seedlings. Thus, in combination with other control measures, seed predation can contribute to limit the growth in population densities of *E. crus-galli*

6. As in-field conditions act as secondary filter on life-cycle transitions seedling survival and fecundity, the seed production per unit area varied between fields. Fields differed in soils nutrient status. In the field with the lowest status, seed production per unit area was lowest. Thus, the conditions in the field affected the growth in population densities of *E. crus-galli.*

Curriculum vitae

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Education

Since 2013	PhD-Student University of Rostock | Germany
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Professional Experience

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2012	Student Assistant International Rice Research Institute | Los Banos | Philippines
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Eidesstattliche Erklärung

Hiermit erkläre ich durch eigenhändige Unterschrift, die vorliegende Dissertation selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet zu haben. Die aus den Quellen direkt oder indirekt übernommenen Gedanken sind als solche kenntlich gemacht. Die Dissertation ist in dieser Form noch keiner anderen Prüfungsbehörde vorgelegt worden.

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